May 1926 Entomological Series Vol. IX, No. 5

# Memoirs of the Department of Agriculture in India

## Experiments on the Transmission of Rinderpest by means of Insects

BY

S. K. SEN, B.Sc., F.E.S.,
Assistant to the Imperial Entomologist

AGRICULTURAL RESEARCH INSTITUTE, PUSA

Calcutta: Government of India
Central Publication Branch
1926

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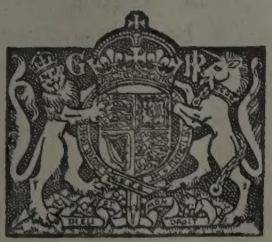

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# EXPERIMENTS ON THE TRANSMISSION OF RINDERPEST BY MEANS OF INSECTS

BY

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*Assistant to the Imperial Entomologist.*

[Received for publication on 1st June, 1925.]

## ERRATA.

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| „ | 183, | „ | 2, | „ annum | „ anum |
| „ | 184, | „ | 9, | „ spiochæte | „ spirochæte |

(1) Rinderpest virus does not survive beyond 24 hours in corrals, bare of vegetation, but containing water.

(2) Animals become infected in such corrals within half an hour, twelve hours and seventeen and one-half hours, respectively, after removal of the sick.

(3) Transmission is possible by close contact only during the febrile period of disease and most certainly during the period in which the temperature is declining. Disease is not contracted in contact with convalescent animals.

(4) Blood was shown infected in two cases during the he ght of febrile period.

(5) The virus in urine, diluted with water and sprinkled on grass, was demonstrated to survive for 36 hours in some instances, but not always, and not for a longer period.

---

[1] *Rev. Gén. Méd. Vét.*, XXXI, pp. 57-60 (1922). [Abstract in *Rev. App. Entom.*, B, X. p. 15, (1922).]

[2] *Philipp. Journ. Sci.*, IX, Sec. B, Tropical Medicine, pp. 49-80 (1914).

(6) Fæces mixed with water and sprinkled on grass infected 24 hours later.

(7) Fæces and urine mixed with water and kept in shade remained infective for 36 hours, but not longer.

(8) There was no evidence that recovered cases transmitted the disease.

(9) Virus is not harboured for long periods in contaminated soil.

(10) Therefore the virus of rinderpest perishes soon after being discharged by the infected animal.

Shilston's observations [1] on the viability of the virus would also appear to be, in the main, in accord with those of Ward and his collaborators, although, so far as the duration of its vitality in meat, bone marrow and blood was concerned, he found it to vary from 3 to 51 days.

In the case of the majority of diseases, in which insect vectors are supposed to be concerned, there does not appear to be on record much evidence of efforts having been made to correlate the epidemiological *data* with the ætiology of those diseases, in so far as such *data* are calculated to throw light on the probable character of the vectors concerned. The mere linking of the seasonal outbreak of a disease with the seasonal prevalence of an insect, can be but of little value, unless the question is viewed from an epidemiological standpoint, *i.e.*, unless the combination of all the circumstances under which an epidemic is found to occur, should point to a particular insect or a group of insects as being concerned in the propagation of the disease. This would require not only an intimate knowledge of the bionomics of the insect that is incriminated, but also a careful sifting of all ecological *data* relating to the question. For instance, Ashburn and Craig [2] advance the following grounds for suspecting mosquitos as the probable transmitters of dengue: "Its seasonal prevalence, its occurrence along low-lying, moist coast regions, and in the valleys of rivers, most frequently; its rapid diffusion in some localities, and its lack of diffusion in others; its relation to changes in temperature and moisture; its manner of spread from building to building in infected places; its absence in high altitudes where mosquitos are absent; the presence of multitudes of mosquitos wherever dengue occurs, and the absence of the disease in regions where mosquitos are absent or few in numbers, and the cessation of the epidemic in badly infected districts when conditions arise which are unfavourable to the propagation of mosquitos, all point to some species of this insect as the infecting agent." In the case of relapsing fevers, again, Todd [3] refers to the fact that these fevers are "characteristically seen in times of stress and where human beings are herded together in unsanitary surroundings." "Relapsing fevers," he says, "are among the plagues of armies. When relapsing fever occurs outside an endemic area, it usually appears in ships, in hotels, or in communities on lines of travel"; and all these facts point to lice as the probable transmitters, and the hypothesis has been borne

[1] *Mem. Dept. Agri. India, Vet. Ser.*, III, No. 1 (October 1917).
[2] *Journ. Infect. Dis.*, IV, pp. 464-465 (1907).
[3] Byam and Archibald, *The Practice of Medicine in the Tropics*, Vol. II, p. 1254 (1921).

out by experimental evidence. The recent observations on the kala-azar epidemic, particularly those of Knowles and his collaborators,[1] are equally instructive. They exclude lice, as the community concerned are not infested to any marked degree by these insects. Mosquitos and other flying hosts with a wide range of distribution are also excluded in view of the localized and household distribution of the disease. *Phlebotomus minutus* and *P. papatasii* are not suspected, as the two species occur all over India, but the distribution of *P. argentipes*, they state, corresponds with that of the disease. On the other hand, epidemiological evidence may sometimes point to a very unexpected conclusion. For instance, on epidemiological grounds, kala-azar in the Sudan was considered by Archibald to be transmitted, not by biting insects, but probably by "some intermediate host, whose habitat is in water."[2] Although, therefore, the results of bacteriological investigations may be calculated materially to help in building up a hypothesis as to the probable character of an insect vector, so far as entomological endeavours are concerned, it would seem expedient to direct them, as a preliminary step, towards compiling all epidemiological *data* bearing on the problem; otherwise, the trouble entailed by experimenting with a large number of species, on the mere chance of securing positive results, is likely to assume enormous proportions.

## II. The cyclical transmission of pathogenic organisms: the intermediary host.

It is useful to remember that the mere fact that an insect can transmit a disease only after a length of time since it sucked infected blood, is not in itself indicative of the fact that the causative organism undergoes a developmental cycle within the invertebrate host, such as is understood by the term "metaxeny." Except in a few cases, such as those of *Plasmodium* and *Hæmoproteus*, where schizonts, sporozoites, etc., have been definitely observed, there does not appear to be on record unequivocal evidence that might justify one to interpret the conditions, under which a disease is transmitted by an insect, as indicating a "metaxenous" mode of transmission, and, under such conditions, the possibility of the insect functioning as a cultural medium for the ingested parasite to multiply, should not be overlooked. Noguchi takes the only correct position when he says in regard to the transmission of yellow fever by *A.* (*S.*) *argenteus*: "The term intermediary host, however, is not used here in the sense understood in the case of certain protozoan organisms, which require an extrinsic host in which to pass their life-cycle, but denotes that a certain length of time is necessary for multiplication of the organisms to such numbers that the mosquito may transmit enough to produce infection. From the biological and cultural properties of the organism this seems

[1] *Ind. Med. Gaz.*, LVIII, pp. 321-349 (1923).
[2] *Report of Adv. Committee for Trop. Dis. Research Fund for 1914*, p. 116 (1915). Cited by Fantham in *Ann. Trop. Med. & Parasit.*, IX, p. 347 (1915).]

reasonable, though the possibility of a stage of development in the mosquito has not been excluded." [1] The results of Noguchi's experiments with *A.* (*S.*) *argenteus* tend to prove that the traditional view, that the mosquito can transmit yellow fever only after a length of at least 12 days since its infective feed, is no longer tenable,—a view about which Seidelin (1911) had already expressed his misgivings. A few more opinions may be quoted, as on a proper appreciation of the issues involved in the two conceptions will depend, to a large extent, when positive results, if any, may be expected to ensue in any series of transmission experiments, and on it will also depend the range of insects to be experimented with; since, in the case of a truly "metaxenous" mode of transmission, the interval between the ingestion of the parasite by the insect and the appearance of the disease in the normal animal bitten, would remain approximately constant, and, also, highly specialized environmental conditions being necessary for the parasite to complete its developmental cycle, the vectors are likely to be restricted to one or a few species of insects; on the other hand, if the insect functions only as a cultural medium for the parasites to multiply, positive results may ensue at any time during the progress of a transmission experiment, it being merely necessary that the parasites should be enough in number to produce the infection, and, consequently, the vectors are likely to extend over many species of insects. The following remark by Noguchi, however, would seem to imply the possibility that only *A.* (*S.*) *argenteus* may provide the necessary cultural medium for *L. icteroides*: "Whether or not *L. icteroides* can survive and multiply only in the body of *Stegomyia calopus* and not in other varieties or genera is yet to be determined."[2]

Swingle [3] would appear to tend to be platitudinous when he remarks, in connexion with *Trypanosoma lewisi*, "After several years of doubt and argument, rigid experiment and accurate observation have brought to definite knowledge the fact that certain invertebrates serve as true intermediary hosts for blood trypanosomes. By the last clause, I do not mean to convey the idea that the trypanosome necessarily passes through a sexual cycle within the invertebrate host. This much has been proved, that it establishes itself in the invertebrate in such a manner as to make possible for an indefinite period its introduction into its vertebrate host by the bite of the intermediary host. In other words, the cyclical method of transmission has been established. This does not annul the fact that the invertebrate may in addition act as a mere mechanical carrier."

---

[1] *Journ. Exper. Med.*, XXX, p. 406 (1919).

[2] *Loc. cit.*, p. 410. *Cf.* Agramonte's observations in a recent paper in this connexion. He argues that in all known cases of insect-borne spirochætal diseases, "the insects act as simple vectors or carriers of the germ, while we have reason to believe that in yellow fever the parasite must undergo some kind of evolution in the mosquitos' tissue, just as the malarial parasites do in the anopheline." He further writes: "I have not heard of any one attempting to demonstrate an insectile evolution of *L. icteroides* in *Ædes aegypti*. An *enriching* process has been suggested, but that is only a hypothesis to explain the occasional success in the midst of many negative experiments. We know that no changes take place, and that the mosquito soon expels the leptospira just as it took it from the capillaries of the guinea-pig."—*Journ. Trop. Med. & Hyg.*, XXVII, p. 286 (Nov. 1, 1924).

[3] *Journ Infect. Dis.*, VIII, p. 125 (1911).

That there is a general tendency to attribute only to such organisms, as bacilli, the property ot multiplying within the invertebrate host (without undergoing a developmental cycle), is exemplified by the following quotation: "Another observation which has been especially puzzling to all investigators has been the fact that the lice seem only to become infective five to six or even ten days after the first feeding. Nicolle believed that this was evidence that the micro-organism is a protozoon, and that this length ot time was needed for it to pass through some developmental cycle in the body of the louse, preliminary to becoming infective. Since the micro organism is a bacillus, the only possible explanation is the one suggested originally by Ricketts and Wilder, that during the period the bacilli in the lice are undergoing an increase in numbers and virulence."[1]

The fact that in some cases the same disease has been proved to be transmitted by more than one species of insect and, in many cases, at various intervals of time, would seem to indicate that true metaxeny is rather the exception than the rule, although in the absence of actual demonstration, any hypothesis advanced in this connexion must be considered as *ex cathedrâ*.

[1] Plotz, H., Olitsky, P. K., and Baehr, G. *Journ. Infect. Dis.*, XVII, p. 62 (1915).

## PART I.

### Transmission of Rinderpest by means of *Aëdes* (*Stegomyia*) *albopicta*, Skuse.

These experiments were suggested by certain bacteriological findings obtained by the Director of the Imperial Institute of Veterinary Research, Muktesar, in the course of his investigations upon the ætiology of rinderpest, which seemed to indicate that the causative organism of the disease was, in many of its characteristics, probably not unlike *Leptospira icteroides*, the spirochæte incriminated with the causation of yellow fever and known to be transmitted by *A.* (*S.*) *argenteus*; it seemed to him therefore that some further evidence concerning the nature of the virus of the cattle disease might be obtained by exploiting the apparent analogy with the human affection, and hence early in 1923 the Imperial Entomologist was approached with a view to deputing trained entomological assistance to elucidate certain possibilities in regard to vector transmission which had already been investigated by workers upon the ætiology of yellow fever.[1]

The experiments of this series were started by the Imperial Entomologist at Muktesar, about the last week of April 1923, and, on his departure from Muktesar, on 15th May 1923, were continued by the writer of this report until they were brought to a close on 28th October 1923.

#### *Arthropods as vectors of spirochætes.*

During recent years several workers have sought to attribute to spirochætes the pathogenic *rôle* in the causation of various diseases, supposed to be transmitted by insects. Several workers have suspected some species of *Leptospira* as the causative organism of dengue fever and, on epidemiological grounds, Chandler and Rice[2] consider *A.* (*S.*) *argenteus* as the probable transmitter of the disease. Transmission experiments were successful in four out of six cases. They conclude that this mosquito can transmit the disease, as it appeared in the Texas epidemic, in from 24 to at least 96 hours after biting a patient, and that it can become infected from patients in various stages of the disease from the first to at least the fifth day. At Braz-

[1] The results of bacteriological investigations, so far obtained, do not warrant a definite statement at this stage, and further work is in progress whilst this report is being written. Reference may, however, be made to the *Report of the Imperial Bacteriological Laboratory, Muktesar, for the two years ending the 31st March, 1924*, where the results of very extensive investigations upon the nature of the virus are summarized very briefly; they will be published soon as a memoir when they have been elaborated in certain particulars.

[2] *Amer. Journ. Trop. Med.*, III, pp. 233-262 (1923). [Abstract in *Rev. App. Entom.*, B, XI, p. 143 (1923).] In this connexion it is of interest that in the case of dengue, Harris and Duval have demonstrated structures culturally which are similar to those demonstrated by the Director of the Muktesar Institute. See *Journ. Exp. Med.*, XL, pp. 817-833 (1924).

zaville, Blanchard and his collaborators [1] observed a form of spirochætosis which bore a close resemblance to infectious jaundice (of which the causative organism is *L. icterohæmorrhagiæ*). They obtained negative results with *Aëdes* (*Stegomyia*), but positive results with *Cimex lectularius*, some of the bugs remaining infective for 38 days. Whittingham,[2] in the case of sand-fly fever, isolated a species of *Leptospira*, indistinguishable from *L. icterohæmorrhagiæ*. Mention need hardly be made of *L. icteroides*, the causative agent of yellow fever, transmitted by *A.* (*S.*) *argenteus*; *Spirochæta recurrentis* the causative agent of European relapsing fevers, transmitted by *Pediculus* and *S. duttoni* and *S. novyi*, the causative agents of African and American relapsing fevers, in which the vectors are ticks, notably *Ornithodorus moubata*.

*The œtiology of yellow fever.*[3]

Finlay (1883-1899) appears to have been the first definitely to set forward the theory that yellow fever is transmitted by *A.* (*S.*) *argenteus*. It was, however, the work of U. S. Army Commission (1901-1902), consisting of Reed, Agramonte, Carroll and Lazear, that was responsible for the general acceptance of the view that yellow fever was transmitted by mosquitos.

According to Carroll (1903) positive results were obtained in fourteen out of twenty-three cases, but evidently in few of the cases were typical results obtained, and this was accounted for by the fact that the patients had the advantage of good treatment, from the beginning of the disease. Positive results also attended the efforts of Guiteras (1901), of Marchoux, Salimbeni and Simond (1903), who composed the famous French Commission on yellow fever, and of others, fifteen positive cases being added, according to Carroll (1905), to the fourteen already obtained by the U. S. Army Commission, although the methods of experimentation employed by these workers, do not appear conclusively to prove that the disease might not have been contracted otherwise than by experimental inoculations.

Finlay (1886) claimed to have obtained positive results by inoculation two days after the mosquito had bitten a yellow fever patient, whereas Reed and his collaborators found an interval of at least 12 days necessary. The French Commission, as pointed out by Seidelin, although usually quoted as the authors of the twelve day doctrine, seem to have drawn their conclusion from the facts published by the American Commission and in their later papers Reed (1902) and Carroll (1905) do not draw any definite conclusion from their own observations, so that, as observed by Seidelin, "it is obvious that it is, at present, impossible to lay down any definite time-limit; nevertheless, it is now everywhere in yellow fever literature taken for granted that the mosquito can only transmit yellow fever after a period of at least

[1] *Bull. Soc. Path. Exot.*, XVI, pp. 184-193 (1923).
[2] *Proc. Roy. Soc. Med.*, XVI, pp. 1-14 (1922).
[3] Many of the references in this section have been taken from Harald Seidelin's article entitled "The etiology of yellow fever" in *Yellow Fever Bureau Bulletin*, I, p. 229 *et seq.* (1911-12).

twelve days since it sucked infected blood ; this is taken for granted, simply because it has been once said, though without any proof, and afterwards repeated again and again. The inference has been made next, and quite naturally, that the yellow fever parasite undergoes an evolution of twelve days' duration in the *Stegomyia fasciata*. This remains to be investigated."

The main conclusions arrived at by the French Commission were :—

1. The serum of a patient is virulent on the third day of the illness.
2. On the fourth day the blood of a yellow fever patient no longer contains the virus, even when the fever is higher.
3. One-tenth c.c. of virulent serum injected under the skin is sufficient to give the fever.
4. As proved by Reed, Carroll and Agramonte, yellow fever is produced by the puncture of *A.* (*S.*) *argenteus*.
5. To be able to produce the illness with man, the mosquito must be infected, previously, by absorbing the blood of a yellow fever patient during the first three days of the sickness.
6. The infected mosquito is dangerous only after an interval of at least 12 days after it has sucked virulent blood.
7. The punctures of two infected mosquitos may bring about a very severe case.
8. The mosquito appears to be more dangerous in proportion with the length of time which has elapsed after infection.
9. The puncture of infected mosquitos does not invariably give yellow fever.

The Commission was unable to find, either in the mosquito or in the blood, the causative organism of yellow fever. [1]

It is out of place in an entomological paper to refer exhaustively to the efforts made by various workers to discover the causative organism of yellow fever. A perusal of the history of the progress of this inquiry shows that the parasite has been identified, at one time or another, with almost every kind of micro-organism. Numerous bacilli and cocci have been described in the belief that they represented the causative organisms of the disease. Reviews of the corresponding literature are found in the treatises of Bérenger-Féraud (1890) and Azevedo sodré and Couto (1901). The causative organism was considered to be *Cryptococcus xanthogenes* by Freire (1885, 1898) ; *Bacillus icteroides* by Sanarelli (1897) and Bandi (1904). Various protozoan-like organisms have also been described as the pathogenic agents of yellow fever, by Klebs (1898), Lacerda (1893), Pothier, Hune, Watson and couret (1905), Schüller (1906) and Thayer (1907). Schüller observed schizonts and gametes and even spirochætoid forms, whilst Stimson (1907) observed a spirochæte in the sections of the kidney in a case of yellow fever. Seidelin also claimed the discovery of the

[1] *Ann. Inst. Pasteur*, XVII, pp. 728-730. (1903).

causative organism of yellow fever, which he considered to be a protozoon. The honour of discovering the actual parasite, however, was reserved for Noguchi, who, by his recent researches, has conclusively demonstrated that the causative organism is *L. icteroides*. A summary of Noguchi's results of transmission experiments is given below :

(*a*) *Transmission experiments with* A. (S.) argenteus *from man to animals.*

Expt. 1. Aug. 5, 1918. 40 engorged on a patient. Aug. 10. 16 females surviving ; these fed on young normal guinea-pig. Negative.

Expt. 2. Aug. 15. 12 of the 16 mosquitos in the above experiment surviving. Aug. 28. 8 still surviving ; these fed on normal guinea-pig. Positive. (Mosquitos had sucked patient on 3rd day of disease.)

Expt. 3. Aug. 5, 1918. 28 females engorged on 4th or 5th day of disease. Aug. 10. 12 surviving. Aug. 12. Mosquitos placed on normal guinea-pig ; several killed by animal and several engorged. Negative. Aug. 28. Surviving females allowed to bite normal guinea-pig ; temperature rose to 39·9°C. but returned to normal.

Expt. 4. Aug. 14, 1918, 4-15 P.M. A large number placed on arm, 2nd day of disease. 60 females fully engorged and put into another cage. Aug. 15. Another feeding on the same patient. Aug. 27. 29 surviving ; these fed on normal guinea-pig. Doubtful (probably mild infection).

Expt. 5. Aug. 14, 1918, 5 P.M. Right arm of patient put into cage on 2nd day of disease. Of 50 mosquitos only 5 sucked within about 30 minutes. Aug. 15, noon. Feeding repeated ; 32 females engorged immediately. Aug. 28, 10 A.M. 13 survive and fed on normal guinea-pig. Negative.

Expt. 6 Aug. 28, 1918, noon. 80 females engorged on a mild case. Sept. 11. 23 surviving and allowed to feed on normal guinea-pig. Negative.

(*b*) *Transmission from animal to animal.*

Expt. 7 Aug. 4, 1918. 50 females infected with blood of guinea-pig which had been infected with *L. icteroides* 6 days previously. Aug. 8. Mosquitos fed on another guinea-pig showing fever and albuminuria. Mosquitos which were used later in the experiment recorded below were allowed to bite normal guinea-pig on the 6th day after first feeding ; only 5 females sucked blood. Negative.

Expt. 8. Aug. 16 (12 days after the first and 8 days after the second feeding). Two normal guinea-pigs placed in cage ; one bitten by 19 mosquitos. Positive. The other bitten by nine other mosquitos on the same day. This guinea-pig remained well for 24 days and was then subjected to a second infection with the same strain of the organism on Sept. 7. It proved refractory to the infection.

Expt. 9 Mosquitos used in Expt. 8 were kept for another experiment, but within 5 days, only 8 of the 28 mosquitos survived. These were allowed to bite another normal guinea-pig on Aug. 21. Negative.

Expt. 10. Aug. 13, 1918. In this series 4 guinea-pigs infected at different stages of the disease, 6th, 7th, 8th and 9th days after the inoculation, all showing the symptoms, were used to infect the mosquitos in four separate cages. 142 engorged females in all were collected and put together in one cage for further experiments.

Aug. 21. 83 females surviving and these fed on normal guinea-pig. Death occurred within 13 days. Positive.

Expt. 11. To supplement the foregoing experiments, on Aug. 23, *i.e.*, 2 days later, the same mosquitos, 25 in all, were crushed in a mortar and emulsified with Ringer's solution. The emulsion was examined for *Leptospira* under the dark-field microscope and also smeared over the scarified surface of the skin of a normal guinea-pig. Occasional specimens of *Leptospira* were found in the emulsion after long search.

The guinea-pig came down with typical symptoms on Aug. 31, *i.e.*, 8 days after inoculation. In the liver and kidney *Leptospira* was demonstrated in small numbers under the dark-field microscope.

Expt. 12. Some of the mosquitos used in Expt. 10, which had caused positive transmission after 8 days, were kept for another work. As stated above, on Aug. 23, 25 were crushed. Subsequently most of the remaining females laid eggs, and some perished. On Aug. 28 only 13 females were left; these allowed to bite a normal guinea-pig on that date. Negative.

Expt. 13. Aug. 11, 1918, 11 A.M. An infected guinea-pig, showing typical symptoms, was placed in mosquito cage; 24 engorged females were collected and put into another cage. Aug. 14. 16 additional engorged females were also put into the cage. Aug. 21. Mosquitos fed on a normal guinea-pig. Probably a mild infection.

*Conclusion.* In the case of human beings a mosquito is said to transmit yellow fever after a period of at least 12 days since it has sucked infected blood, but in the case of the animals the period was only 8 days. This difference can be explained by the fact that the number of *Leptospira* in experimentally infected animals is far greater than in naturally infected human beings.[1]

## I. Methods and material.

### *The selection of the invertebrate host.*

*Aëdes* (*Stegomyia*) *albopicta* was the mosquito selected for these experiments on account of the great abundance of this species in India, and also in view of the fact that both in morpho'ogy and habits it is very like the yellow fever mosquito,

[1] *Journ. Exper. Med.*, XXX, pp. 404-409 (1919).

*A.* (*S.*) *argenteus.* As a matter of fact, Leishman even planned to carry out a series of experiments with *A.* (*S.*) *albopicta* in connexion with yellow fever.[1]

In none of the experiments were wild mosquitos used, all being bred out under laboratory conditions.

### *The place of experimentation.*

Muktesar, the place where the experiments were carried out, is situated at an altitude of about 7,500 ft. The minimum and maximum temperature of the place during the summer months is given in Appendix II. The two great advantages of the place were the existence of a well-equipped laboratory and the presence of a large number of experimental animals, an experimentally inoculated animal being obtainable almost whenever required.

Kinghorn and Yorke,[2] in an article entitled "On the influence of meteorological conditions on the development of *Trypanosoma rhodesiense* in *Glossina morsitans*," write: "The developmental cycle of *T. rhodesiense* in *G. morsitans* is, to a marked degree, influenced by the temperature to which the flies are subjected. High temperatures (75°-85° F.) favour the development of the parasite, whilst low temperatures (60°-70° F.) are unfavourable." If this is also true of the spirochætes, then the parasites (if these belonged to that group) must have been at a disadvantage in the very low temperature of Muktesar.

### *The selection of the reservoir of virus.*

For the initial feeds the mosquitos were allowed to bite bulls experimentally inoculated with the virus of rinderpest, such infected bulls being called "Controls."

It was somewhat difficult to come to a decision as to the best time for the mosquitos to take their initial feeds, during the progress of the disease in experimentally inoculated animals. Curasson (*loc. cit.*) removed his tick on the second day of the disease, but in view of the very inconclusive character of his results, it is not possible to say anything for or against his choice. Noguchi fed his mosquitos (*loc. cit.*) on from the second to the fifth day of the disease in human beings. Reed (1902) concludes from experiments with direct inoculation of blood that the specific agent of yellow fever is present in the blood at least during the first, second and third day of the attack. Marchoux, Salimbeni and Simond (1903) deduce a similar conclusion from their blood injection experiments. After very exhaustively considering the *data* from which these workers have drawn their conclusions, Seidelin remarks: "It can, consequently, not be considered proved that the blood of a yellow fever patient is infective during the first three days only; much less can it be concluded that the parasite must be absent from the blood after that time."

[1] White is of opinion that "it is possible that *Aëdes albopicta* (*s. vittellaris*) is a vector as well as *A. argenteus* (*aegypti*)." *A Folha Medica*, V, pp. 193-197 (1924). [Abstract in *Rev. App. Entom.*, B, XII, pp. 173-174 (1924).].

[2] *Ann. Trop. Med. & Parasit.*, VI, p. 412 (1912).

In the present series of experiments the time just prior to, or immediately following, the onset of vesicles on the buccal mucous membrane, was usually selected for the initial blood feeds, although in some cases this rule was not strictly adhered to.

*The selection of experimental animals.*

The normal bulls on which the infected mosquitos were fed were usually kept singly in small sheds, called "*chuppers*." These sheds were situated fairly wide apart from one another, and at a considerable distance from the shed where the "Controls" were kept. In view of the preliminary character of the experiments aimed at, no attempt was made to protect the experimental animals from the bites of other insects.

O'Brien refers to a suggestion, made by Dr. Pareja of Guayaquil, that children are less susceptible to contract yellow fever than adults owing to the fact that the liver in children is larger in proportion to the body than is the case in adults, and "it may have some function or secretion absent in the adult, and which has the power of combating the yellow fever virus."[1] O'Brien remarks that "if this property of children is common to other young mammals, it might have its use either as a protective or as a curative agent."[1] The series of experiments recorded in this paper were, however, carried out on both adult and young animals.

*The rearing of* A. (S.) albopicta in high altitudes.

Tree-hole materials, containing *Stegomyia* eggs in desicated condition, were taken from Pusa, the materials being divided into two lots, labelled I and II. The first represented the comparatively loose and superficial débris, the second being obtained by scraping the sides and the deeper regions of the hole. As will be seen from the schedule of breeding experiments, the two lots differed considerably in the richness of materials (eggs).

Later on, the tree-hole materials taken from Pusa being exhausted, attempts had to be made to get eggs deposited in captivity at Muktesar, and the results of these attempts are also given in this paper.

As the very low temperature of Muktesar retarded the matamorphosis of the various stages and considerably delayed oviposition, the use of an incubator, kept at about 28° C., was afterwards resorted to.[2]

The tree-hole materials taken from Pusa, although yielding well-sized adults when treated with water did not appear to provide sufficient nutriment when used a second time for the larvæ that hatched out from eggs deposited in captivity at Muktesar, and therefore other sources of food had to be looked for.

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[1] *Ann. Trop. Med. & Parasit.*, VIII, p. 375 (1914-15). The Director of the Muktesar Institute also observes that recent experiences at Muktesar point to a greater resistance of the young animal towards rinderpest.

[2] 28°C. was the temperature chosen in view of the findings of the French Commission with regard to *A.* (S.) *argenteus*.

The uncertain nutritive value of various algæ and leaves (some of which might even be toxic to the larvæ), coupled with the difficulty of procuring Stegomyias at Muktesar, made it necessary to try only such food as had been tested and found satisfactory by previous workers.

Reed and Carroll and also the French Commission on yellow fever found that *A.* (*S.*) *argenteus* throve remarkably well in water containing fæcal matter, whilst Dupree and Morgan record that they found that it even hastened the development until the life-cycle was completed in from six to eight days. The French Commission also report having reared *argenteus* larvæ easily in water in which had been placed excremental pellets or grains of corn or wheat, alimentary débris and amylaceous and fatty material.[1]

Atkin and Bacot,[2] Fielding,[3] and Young[4] have tried a number of substances as foods for *Stegomyia* larvæ, and Fielding, particularly, has even carried out quantitative experiments, giving weighed quantities of the food-material to a known number of larvæ, in measured quantities of water. The materials which he tried were, principally, polished rice, dry cockroach and dry mango leaves. Blacklock[5] also recommends dry cockroach.

In feeding the larvæ of *A.* (*S.*) *albopicta*, particular attention was given to two substances, namely, rice grains and dry specimens of *Musca* sp.,[6] the latter being considered a good substitute for dry cockroach. The chief disadvantage of using rice grains—although otherwise they proved fairly satisfactory—was that, when left in water for a long time in the incubator, they became semi-boiled and showed tendency to ferment; the water smelt rancid and the larvæ did not thrive well under such conditions. Except for the fact that the dry specimens of *Musca* sp., would not sink readily in water—and the sinking was very necessary in view of the fact that *Stegomyia* larvæ are chiefly bottom-feeders—they answered well as food for the larvæ, and mortality was almost *nil*.

### *The method of keeping mosquitos and feeding them on animals.*

A considerable mass of literature was gone through with a view to finding out a suitable method of keeping and feeding mosquitos and other biting flies, but the various methods recommended appeared to be unsatisfactory in one way or another. One notices with surprise the perfunctory character of the methods employed by most

[1] Howard, Dyar and Knab. *The Mosquitoes of North and Central America and West Indies*, Vol. I, p. 287 (1912).

[2] *Parasitology*, IX, pp. 482-536 (1917).

[3] *Ann. Trop. Med. & Parasit.*, XIII, pp. 259-296 (1919).

[4] *Ann. Trop. Med. & Parasit.*, XVI, pp. 389-406 (1922).

[5] *Ann. Trop. Med. & Parasit.*, XV, p. 476 (1921).

[6] Specimens of this species were very common in houses at Muktesar. It was curious to notice innumerable dead specimens hanging on walls and on the glass panes of doors and windows, during the summer and monsoon months at Muktesar. On examination it appeared that the flies attached themselves to those places by means of their proboscides, which, evidently, secreted a sticky material when the flies died. Most of the flies were presumably killed by the fungus *Empusamuscæ*.

workers in feeding insects in transmission experiments. The result has been that the validity of their conclusions has not only been questioned by critics, but the conclusions have frequently been half-heartedly upheld by the workers themselves. This state of affairs appears to be, to a large extent, due to the fact that an enormous amount of patience and trouble is required in maintaining and feeding insects at regular intervals of time and, to a partial extent, to the fact that, as many of these workers were *bonâ fide* bacteriologists or protozoologists, emphasis on those aspects which relate to their own special subjects, has resulted in summary work being done with regard to the entomological side of a question. Noguchi himself thus writes of his yellow fever transmission experiments : "Since an enormous amount of labour would be required to test the infectivity of each female mosquito, summary experiments were made ; that is, a dozen or more mosquitos were allowed to bite one and sometimes two animals at the same time" (*loc. cit.*, p. 404). Curasson (*loc. cit.*) remarks on his own experiments that as conditions with regard to isolation, during the experiments were not above suspicion, no great importance is attached to the positive results obtained. The same remarks apply to the yellow fever transmission experiments carried out by Finlay, Reed and others. Regarding the dengue fever transmission experiments, carried out by Guiteras and Cartaya, Guiteras himself remarks that the small number of the mosquitos and their lack of variety deprive the negative result of a claim to conclusive character.[1] Again, Agramonte, in spite of his failure to transmit dengue by means of mosquitos, states that " he believes that the mosquito transmits the disease, and that his negative results were due to some undiscovered fault in technique."[2]

In attempting to transmit *Trypanosoma gambiense* by various flies, Dutton and his collaborators kept them in cubical cages, eighteen inches along each side and containing water and growing grass, the cages being placed in sheltered position out of doors. At first the experimental animals were set free within the cages, but afterwards they were immobilized. " The method, however," as they confess, " had distinct disadvantages : it was difficult to feed the flies on large animals and, because of the size and contents of the cages, it was almost impossible to count the number of living flies they contained."[3] The method adopted by Kinghorn and Yorke[4] for feeding tsetse flies appears to have been sound in view of the protection it afforded against outside contamination. They enclosed the animals in fly-proof cages, the fronts of which were protected by a double layer of wire-gauze, the inner composed of coarse and the outer of mosquito meshing. The two layers were separated by a space of one inch in order to obviate the possibility of the animal being bitten while

[1] Cited by Ashburn and Craig in *Journ. Infect. Dis.*, IV, p. 442 (1907).

[2] Cited by Ashburn and Craig. *Ibid*, p. 442 (1907).

[3] *Ann. Trop. Med. & Parasit.*, I, p. 203 (1907-08). Amongst the insects they experimented with, were Anophelines and *Simulium*, but with regard to the latter, they write : " It was hoped to do a series of experiments with them, but they were found to be so difficult to manage that the idea was given up" (p. 215) ; some experiments with freshly caught specimens, however, gave negative results.

[4] *Ann. Trop. Med. & Parasit.*, VI, pp. 3-4 (1912).

pressing the body against the front of the cage. Each fly was preserved in a separate bottle and had special number, so that an exact history of its life, the number of meals it had, the animals on which it fed, and other particulars were available. As will be seen from what follows, a similar method was adopted for feeding *A* (*S.*) *albopicta*, although for obvious reasons the experimental bulls could not be kept in the way in which Kinghorn and Yorke kept their monkeys. In attempting to transmit poliomyelitis by the bite of *Lyperosia irritans*, Francis[1] used a big cage, the top of which was made of wire-gauze, over which the monkeys, after having been shaven on the belly and inside of the thighs, were kept stretched in order to allow the enclosed mosquitos to bite through the meshes of the wire-gauze, but he writes that "great difficulty was experienced in keeping the flies alive in captivity. If left unfed for twenty-four hours practically all of the flies die. It was found that frequent feeding on a live animal was necessary. The plan finally adopted was to allow them to bite a monkey every six hours." In the case of *Glossina palpatis*, Stuhlmann found that it was best to keep the flies singly or few together, otherwise they fouled one another with excreta. Therefore he used glasses, the mouths of which were covered with mosquito netting, through which the flies sucked. He recommends the use of blotting paper for lining the glasses in order that the excrement may be absorbed and the glasses be kept clean.[2] Mitzmain[3] made an effort to induce *Tabanus striatus* to feed on healthy and infected animals kept together in a large screened cage, but the results were negative, the flies dying in a few days when kept within the enclosure. Cross and Patel[4] kept their Tabanids in cloth cages over basins of water in such a way that the flies could suck moisture whenever they wanted; the flies were taken out in small beakers for feeding, and placed back into the cage when the feeding was over.

With regard to the keeping and feeding of mosquitos in particular, Fielding[5] recommends a cloth cage to enclose the mosquitos; after the animal is immobilized, its head is inserted into the cage through a door in order to allow the mosquitos to bite, but he says that the feeding of mosquitos on small animals (*e.g.*, guinea-pigs) is, as a rule, unsuccessful. Ashburn and Craig[6] used mosquito-proof tents and mosquito bars for dengue fever transmission experiments with mosquitos. Stephens and Christophers[7] recommend keeping and feeding mosquitos in bottles. Leese,[8] who used wild mosquitos, recommends feeding them singly in tubes, as otherwise it is frequently difficult to vouschafe that a mosquito has actually bitten. Patton and Cragg[9] recommend feeding them in bottles, and, in the case

[1] *Journ. Infect. Dis.*, XV, pp. 1-5, fig. 2 (1914).
[2] *Bull. Sleeping sickness Bureau*, I, p. 33 (1908).
[3] *Philipp. Journ. Sci.*, Sec. B, VIII, p. 226 (1913).
[4] *Dept. Agric. Punjab Vet. Bull.*, No. 5 (1921).
[5] *Ann. Trop. Med. & Parasit.*, XIII, pp. 260-261 (1919).
[6] *Journ. Infect. Dis.*, IV, p. 466 (1907).
[7] *The Practical Study of Malaria and other Blood Parasites*, pp. 93-94 (1908).
[8] "Experiments regarding the natural transmission of surra carried out at Mohand in 1908." *Ind. Civil Vet. Dept. Mem.*, No. 2, p. 15 (n.d.).
[9] *A Text-book of Medical Entomology*, pp. 269-270 (1913).

of mosquitos that will not feed under such conditions, they suggest the use of mosquito curtains. Price[1] gives a very detailed description and illustration of a cage which he used with success for rearing and keeping mosquitos its special feature being the adjustability of its component parts and it was provided with a movable glass top with a door for the insertion of the hand. Darling[2] considers that ordinary glass chimneys, covered at both ends with netting, are suitable both for keeping and feeding mosquitos. Pressat[3] used an elaborate cage the chief feature of which was a metallic chimney into which was fitted a removable glass tube. In order to take out the mosquitos, disturbance was caused amongst them by means of a sort of small broom-stick (" chasse-mouches en fibres de palmier ") and the mosquitos were thus made to enter the glass tube, which was then removed. Blacklock[4] recommends for the larvæ the use of small tubes provided with special lids, the tubes being placed in a row in grooves cut into a wooden platform. Noguchi's cage consisted of a wooden frame with a wire net on all but two opposing walls, which were solid pieces of wood with round opening in the centre for the insertion of the arm or a glass of water. The opening was closed by a well-fitting door which could be drawn to one side and slipped back in place. A long glass cylinder about 8 inches long and 2 inches in diameter with one end closed was used for handling individual mosquitos (*loc. cit.*, p. 402).

It is outside the scope of the present paper to enter into a detailed consideration of the methods enumerated above, but they may be briefly dealt with, inasmuch as on the nature of the technique employed depends, to a large measure, the success in transmission experiments.

In the first place, the method to be adopted will depend on whether it is intended to feed the insects in batches or singly. In either case it is essential that a large number of insects should be fed, in view of the fact that in the majority of insect-borne diseases, only a small percentage of the insects fed on infected animals becomes infective.[5] For example, Kinghorn and Yorke[6] found that only about 5 per cent. of tsetse flies (*Glossina morsitans*) became infected when fed on animals harbouring the trypanosomes. Bruce[7] found the percentage to be only

[1] *Journ. Infect. Dis.*, supplement No. 3, pp. 123-127, fig. 1 (May 1907).
[2] Patton and Cragg. *Loc. cit.*, p. 269.
[3] *Le Paludisme et les Moustiques*, pp. 79-81, f. 4 (1905).
[4] *Ann. Trop. Med. & Parasit.*, XV, pp. 473-477 (1921).
[5] This is considered by some to be due to the ingested parasite not being able to adapt itself to the conditions furnished by the alimentary system of all the individuals of a species of insect; in other words, there is what may be called individual adaptability. Schaudinn considers that this may be due to (1) the insect itself suffering from some infection; (2) inability to digest the ingested blood; (3) an acquired or natural immunity resulting in the death of the specific parasite. [Cited by Ashburn and Craig. *Journ. Infect. Dis.*, IV, p. 468 (1907).] In the case of *A.* (*S.*) *argenteus*, Noguchi argues as follows: " A female *Stegomyia* may take up 0·01 c.c. of blood or even less. Apparently a mosquito occasionally becomes infectious by taking up the one or two organisms which happen to be circulating in the peripheral blood of man, and it is these occasionally infected few which carry the disease " (*loc. cit.*, p. 409).
[6] *Ann. Trop. Med. & Parasit.*, VI, p. 22 (1912).
[7] The Croonian lectures on trypanosomes causing disease in man and domestic animals in Central Africa. *Lancet*, p. 5 (July 3, 1915).

0·2 in the case of *G. morsitans* caught in areas infected with *T. brucei*. Robertson[1] found that 3 to 15·5 per cent. of *Glossina palpalis* became infective when fed on animals harbouring *T. gambiense*. Minchin and Thomson[2] state that about 26 per cent. of their fleas was infected with swarming *T. lewisi*. The French Commission on yellow fever remark : " The puncture of infected mosquitos does not always give yellow fever." Noguchi obtained positive results in only 1 in 6 of his experiments, about 30 to 80 mosquitos being used in each experiment.

Massive feeding need only be resorted to when it is definitely intended that a large number of infected insects should *simultaneously* bite the experimental animal ; in other words, when infection is considered to depend on the number of parasites injected into the vertebrate host at a given time ; for example, Wilder[3] states that in the case of typhus fever " the minimum number of lice found necessary to infect a monkey was 17." Otherwise, although a considerable saving in time may be effected if insects are fed *en masse*, the disadvantages of such feeding are numerous, especially if it is necessary to carry on a large number of feedings at short intervals of time, with the same series of insects. The following are the chief disadvantages of feeding mosquitos in batches, in bottles, at short intervals of time :—

1. There is absolutely no means of knowing the number of times any particular mosquito has fed. Since it is considered that only a small percentage of infected insects can become infective, it is essential to know whether a sufficient number of mosquitos has bitten, in order that a percentage may be worked out.

2. In attempting to bring a particular unfed mosquito to a desired position (*e.g.*, by tapping the bottle), violence is unavoidably applied on those that have fed.

3. When several mosquitos freely move about in a bottle, it is frequently difficult to ascertain how many of them have actually taken their feeds.

4. It is not convenient to feed the mosquitos with foods other than blood, *e.g.*, raisins, bananas, sugar, etc.

5. It was repeatedly observed that even when the enclosed mosquitos had been made to settle on the muslin cover of the tube, they left the muslin immediately the bottle was brought in contact with the skin of the animal. This appeared principally to be due to the sudden difference in illumination caused by the neck of the bottle forming a shadow on the muslin. In a thin-walled, narrow, straight glass tube (in which, however, more than one mosquito can hardly be comfortably accommodated), there is hardly any appreciable difference between outside and inside illumination when the tube is brought in contact with the skin of the animal.

6. It is extremely difficult to control the mosquitos, which usually lie scattered about in the bottle, frequently sheltering behind the piece of cardboard that is kept within the bottle for the mosquitos to rest upon.

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[1] *Phil. Trans. Roy. Soc.*, Ser. B, CCIII, p. 161 *et seq* (1913). [Cited by Hoare in *Parasitology*, XV, p. 413 (1923).]
[2] *Quart. Journ. Micro. Sci.*, LX, p. 485 (1915).
[3] *Journ. Infect. Dis.*, IX, p. 92 (1911).

Wire-gauze or cloth cages have the following disadvantages, in addition to some of the ones enumerated above :—

(1) It is almost impossible to bring any kind of physical pressure to bear upon the mosquitos in order to make them bite, unless one has recourse to contrivances of the kind of Pressat's cumbrous " chasse-mouches."

(2) They are not suitable for feeding on large animals, such as bulls.

(3) If the insects are frequently taken out of the cages for feeding, their liability to injury is considerable and the chances of their escaping are many. Their liability to injury is obvious if the experimental animal is placed within the cage. Several of Noguchi's mosquitos were killed by the guinea-pig moving about in the cage (*vide* his Expt. 3 *ante*).

After numerous trials the final method adopted for keeping the mosquitos was to enclose them singly in 30c.c. Nessler tubes (Fig. 1), each mosquito being numbered, so that a complete record of its history could be maintained, from the date of its emergence till its death.

During the earlier part of the work, moisture was supplied to the mosquitos by keeping the tubes, containing them, inverted over a wet piece of muslin, spread on the bottom of a large beaker. But the disadvantage of this method was that the muslin of the tubes always remained wet, and this evidently caused discomfort to the enclosed mosquitos, which were frequently seen brushing the moisture off their legs or cleaning their sodden wings. Later on, therefore, a piece of wire-gauze was placed over the muslin, in order to prevent the tubes from coming in actual contact with the moist muslin (Fig. 7). It appeared, however, that even under such conditions the mosquitos did not look happy and mortality was rather high, and this appeared chiefly to be due to the fact that the incessantly emanating effluvium from the moist muslin had a deleterious effect on the mosquitos. This fact, coupled with the obvious inconvenience of carrying about the tubes from *chupper* to *chupper*, caused the apparatus, shown in Fig. 8, to be improvised. It consisted of a cubical wooden box, provided with a perforated sheet of wood (Fig. 8*b*) and a zinc tray (Fig. 8*c*). The zinc tray was designed to contain the moist muslin and was provided with a wire-gauze lid (Fig. 8*d*). The tray could be taken out, through a small door at the bottom of the box (as shown), whenever it was necessary to replenish the moisture. The box was designed to accommodate 25 Nessler tubes. A special advantage of such a box was that, being made water-tight, it afforded sufficient protection to the mosquitos against getting wet while being carried about from *chupper* to *chupper*, during the heavy rains of the monsoon months.

It has been recorded that a netting of at least 18 meshes to the linear inch is necessary to prevent *A.* (*S.*) *argenteus* from escaping.[5] With regard to *A.*(*S.*) *albopicia*, numerous trials were given to an assorted series of netting with a view to finding

[5] *Vide* chapter on yellow fever by Carter, in Byam and Archibald's *The Practice of Medicine in the Tropics*. Vol. II, p 1250 (1921); also, Vol. I, p. 27 (where, however, it is stated that a mesh of at least 16 holes to the linear inch should be chosen).

## EXPLANATION OF PLATE XV.

FIG. 1. Nessler tube for keeping and feeding mosquitos.

FIG. 2. Tying up raisin on to muslin.

FIG. 3. Raisin as kept tied up inside Nessler tube: view of interior.

FIG. 4. Raisin as kept tied up inside Nessler tube: view of exterior.

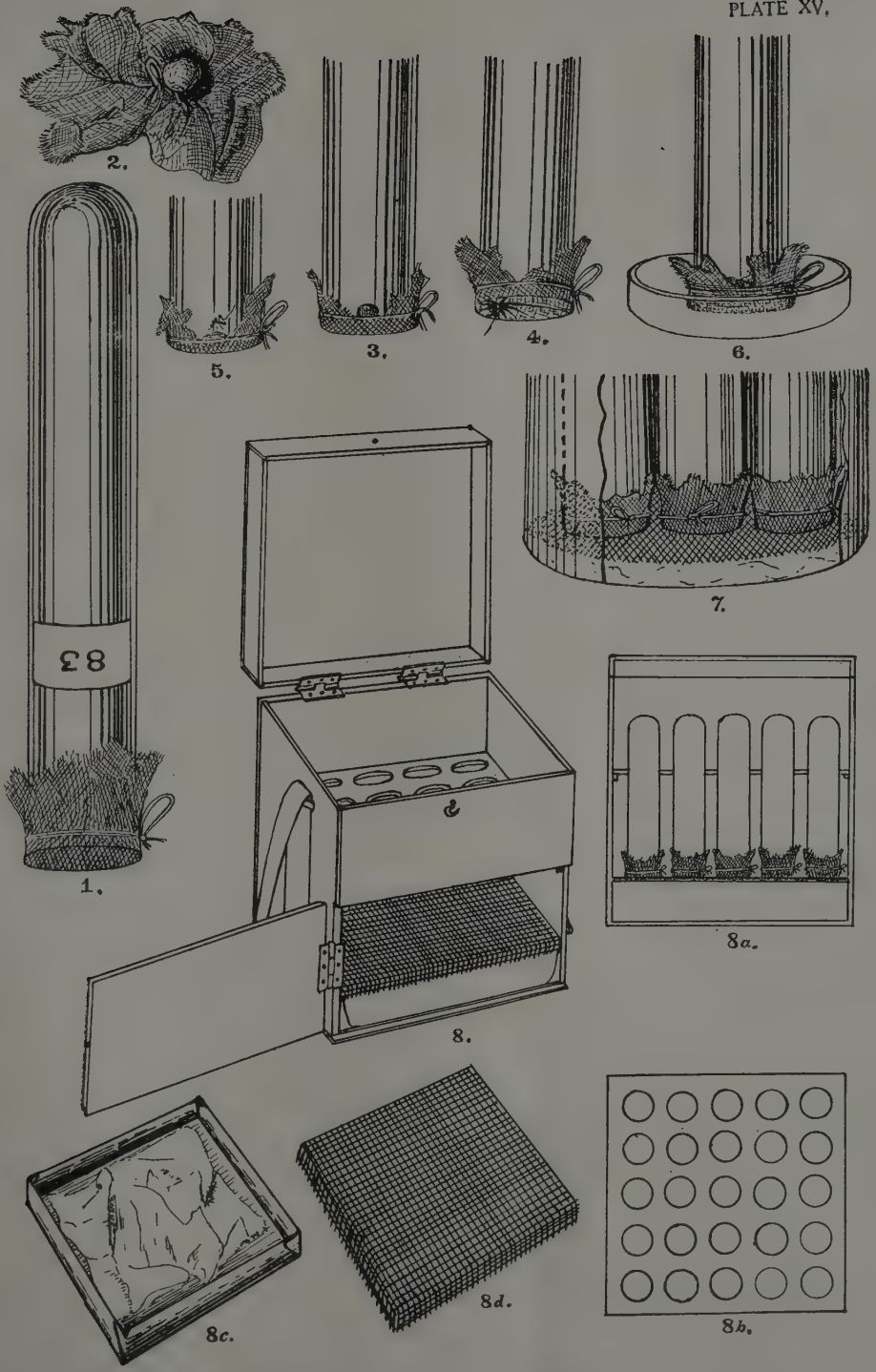
The place where the raisin is tied up is shown by the arrow-head.

FIG. 5. Shows the manner in which the muslin gets drawn inside the tube as a result of the muslin being continually pressed against the skin of the animal during feeding work, so that the skin is hardly within the reach of the mosquito's proboscis.

FIG. 6. Feeding mosquito on sugar-solution.

FIG. 7. Keeping Nessler tubes (containing mosquitos) in beaker.

FIG. 8. Box for holding Nessler tubes containing mosquitos:
*a*, vertical section of box; *b*, perforated sheet of wood; *c*, zinc tray with muslin spread on bottom; *d*, wire-gauze lid for zinc tray.




Showing method of keeping and feeding mosquitos.
(Rinderpest Transmission Experiments)

out the one most suitable for this kind of work. Through the courtesy of the Director of the Imperial Institute of Veterinary Research, inquiries were made both locally and in Calcutta for netting of the requisite grade. It was repeatedly observed that even gorged individuals of *A.* (*S.*) *albopicta* easily escaped through netting, about 13 meshes to the linear inch, such as is ordinarily used for mosquito curtains; and as no finer netting could be procured, the use of muslin had to be resorted to; and here again, repeated trials brought out the fact that, for successful work, it was not enough if the muslin were of the requisite meshing, but it appeared to be necessary to give particular attention to the grade of the thread used; for the disadvantage of thick or loosely spun threads (such as are used in the stuff locally known as *gara* or *khaddar*) is not only that they do not dry sufficiently quickly, when, for instance, the muslin has received a dipping in sugar solution, but, also, that it is frequently difficult to keep the muslin stretched to the extent necessary to ensure successful feeding. This is further discussed later on.

At first, the mosquitos were given meals of sugar solution for their initial feeds and also between feeds of blood. A thin layer of a concentrated solution of sugar was spread in a petri dish and the enclosed mosquitos were brought in contact with the sweet liquid (Fig. 6), when they sucked very readily through the meshes of the muslin. A few dippings in clean water generally removed any trace of the sugar solution that adhered to the mouth-region of the tubes or to their muslin coverings, and, except for occasional discomfort caused by minute quantities of the sugar solution sticking on to the legs (and sometimes to the wings) of the mosquitos, they seemed to remain usually otherwise uninjured. In view, however, of the obvious disadvantages of this method and of the possibility of the cane sugar having a deleterious effect on *Leptospira*, sugar was substituted by raisins, which were tied on to the muslin and offered to the mosquitos in the manner shown in figures. When it was intended to induce a mosquito to bite without removing the raisin, the latter was usually tied close to the wall of the tube, in order that a sufficient area might be left in the muslin on one side for the mosquito to settle upon (Fig. 4).

In the actual work of feeding the mosquitos on bulls, it was repeatedly brought out that the forcible establishment of contact between the mosquito and the skin of the animal was the first condition on which quick and successful feeding depended; for, if the mosquito was away from the muslin, it would seldom migrate of its own accord towards the muslin in order to bite. To induce the mosquito to settle on the muslin, the most effective plan appeared to be to turn the mouth-end of the tube towards the light when the mosquito readily settled on the muslin; if it did not move readily, a few smart strokes on the tube were usually enough to make it do so. It is obvious that for this purpose the most convenient plan would be to admit light from one direction only (preferably from the left) into the room in which the feeding is done. Feeding work done in the open, or in rooms equally illuminated on all sides, was, as a rule, unsuccessful.

Mention must also be made of the necessity of keeping the muslin of the tube as fully stretched as possible by tightly securing it with several turns of a twine. it was frequently observed that, as a result of constant pressure against the body of the animal, the muslin got loosened, its central portion being drawn inside the tube (Fig. 5), with the result that the proboscis of the mosquito could hardly reach the skin of the animal.

Finally, for feeding on bulls—a work requiring extremely arduous and sustained efforts—the necessity of adequately immobilizing the animal need hardly be emphasized. For this the best plan appeared to be to throw down the animal and secure it by tying up the legs with as many turns of a strong rope as possible. Failure to pay adequate attention to this important detail caused the writer many a bitter experience, especially when the animal happened to be a refractory one.

Several workers have referred to the difficulty of inducing insects to bite. Sergent and his collaborators [1] were compelled to content themselves by applying on scarified skin extracts of crushed *Phlebotomus*, having failed to induce these insects to bite. Baldrey[2] states, in connexion with his experiments with *Tabanus* and *Stomoxys*, at Muktesar, that he found it difficult to induce the insects to feed often. Stephens and Christophers (*loc. cit.*, p. 94) recommend moistening the skin of the animal with water in order to induce mosquitos to bite, whilst Connal and Connal[3] found that, in the case of *Chrysops*, moistening the skin of the animal with a drop of sugar solution was effective. The application of simple moisture or of sugar solution did not, however, seem appreciably to influence the biting propensities of *A.* (*S.*) *albopicta*. It appeared, however, that a mosquito would not usually take its first blood feed, unless previously fed on some sweet substance; but for the subsequent feeds of blood, the maintenance of contact of the mosquito with the skin of the animal was found to be all that was necessary, if the mosquito would feed at all.

Although it was usually easy to mark off an engorged mosquito, it was not always easy to trace the imbibed blood in the case of a mosquito which had fed slightly, or a mosquito whose previously ingested food had not been assimilated, or one which had not voided the thick pabulum that usually remains within the abdomen for some time, apparently after the work of assimilation is over. In all such cases a very careful examination with the lens was found necessary, and when blood was detected, it was generally found in the anterior end of the gut. It is obvious that accurate observations of this kind would have been hardly possible if the mosquitos were kept in batches in cloth cages or bottles, and, under such conditions, one would necessarily content oneself by following the method adopted by Bruce and his collaborators in the case of tsetse flies,[4] namely, to reckon only those as having fed, which fill themselves.

[1] *C. R. Acad. Sci.*, CLXXIII. pp. 1030-1032 (1921).
[2] *Journ. Trop. Vet. Sci.*, VI, p. 271 (1911-12).
[3] *Trans. Roy. Soc. Med. & Hyg.*, XVI, pp. 64-89 (1922).
[4] *Bull. Sleeping Sickness Bureau*, I, p. 179 (1909).

The main observations with regard to the feeding habits of *A.* (*S.*) *albopicta* may be summarized as follows :—

1. Contact with the skin of the animal generally furnished the necessary stimulus to the mosquito to bite.

2. Puncturing was not necessarily followed by the drawing of blood. Frequently, a mosquito was seen to stab the skin of the animal or even to keep its proboscis buried therein for a length of time, but careful examination did not reveal any trace of blood in its alimentary canal.

3. A mosquito would bite irrespective of the fact when it had had its last feed,[1] and the extent to which its abdomen was distended was not found to be the criterion of the avidity with which it would feed.

4. Individuals kept without food for a long time showed much reluctance to bite,—a fact which was contrary to expectation.

5. Striking results were obtained when, instead of persisting in one continuous attempt to feed a mosquito, the attempt was repeated at short intervals of time.

6. If interrupted during its first feed, a mosquito usually fed readily when tried a second time immediately after.

7. From the manner in which a mosquito sometimes behaved just prior to the act of biting, it seemed that its sense of vision hardly played any part in enabling it to locate its host. It was frequently observed (*e.g.*, in the case of Mosquito 18), when trying a mosquito on rabbits, that, in spite of the fact that its contact with the animal had been established, it went on moving its proboscis along the edges of the tube as if in search of something, until it (the proboscis) chanced to touch the skin of the animal.[2]

8. Rabbits seemed to be bitten much more readily than bulls.

9. A mosquito sometimes took a full feed only a few hours before death.

10. Permanent mutilation, such as the loss of a leg or a wing, or the presence of adhesive substances like sugar solution, or of even simple moisture, on the legs or wings, *i.e.*, whatever stood in the way of unobstructed locomotion, materially interfered with the biting of *A.* (*S.*) *albopicta*. On the other hand, mosquitos completely deprived of the power of locomotion or otherwise seriously injured, were found, although on rare occasions, to be capable of taking full feeds (*e.g.*, in the case of Mosquito 5 fed on bulls), when kept in prolonged contact with the skin of the animal.

11. Even at as low a temperature as 13°C., mosquitos sometimes bit as soon as their contact with the animal had been established. This observation is of some importance in view of the statements made by various workers with regard to the

[1] It should be mentioned that the interval that was allowed to pass between two feeds was never less than 24 hours.

[2] This seems to support the conclusion arrived at by O'Hea with regard to what he calls the "tactile vision" of insects. (*Nature*, No. 2789, p. 498; April 14, 1923. Also, his article entitled "Vision and light sensitiveness," *Nature*, No. 2795, p. 705; May 26, 1923.)

biting propensities of *A.*(*S.*) *argenteus*. For example, the French Commission found that *A.*(*S.*) *argenteus* became sluggish and would not bite at a temperature below 15°C., and that, at temperatures of from 14°C. to 18°C., it refused to bite when placed in contact with the skin, and that, under such conditions, it would bite only when the temperature inside the tubes was raised by the heat of the hand. Reed and Carroll found 62°F. (15·5°C.) to be the minimum temperature at which *A.*(*S.*) *argenteus* would bite. In the course of the present series of experiments it was brought out that its contact with the skin of the animal usually enabled the mosquito to overcome its torpidity caused by a prolonged exposure to a low atmospheric temperature, provided, of course, it had not been rendered moribund thereby.

12. Confinement in incubator (kept at 28°C.) for 24 hours or more, prior to feeding, did not appear to influence the biting propensities of *A.*(*S.*) *albopicta*, since, although showing considerable activity so long as kept in the incubator, the mosquitos became sluggish as soon as they were brought out and exposed to the low atmospheric temperature.

13. [1]No discrimination could be made as to the degree of readiness with which *A.*(*S.*) *albopicta* would bite between 9 A.M. and 5 P.M., during the months June to October (attempts to feed them at night were not made), and the hour at which it would feed did not seem to bear any relation to its age. These observations are of some interest in view of the experiences of various workers with *A.*(*S.*) *argenteus*. For example, Durham found that the time of chief activity of the mosquito was in the middle of the day, from about 12 noon to 2 P.M. Dutton observed that *A.*(*S.*) *argenteus* bit more especially in the early part of the afternoon, and, according to Hinds, *A.*(*S.*) *argenteus* is more active from 8 to 10 in the morning and from 4 to 6 in the afternoon. The French Commission observed that " when the female has reached a certain age, at the most two weeks, it will under no circumstances bite in the day time ".[2] Carter's observations seem to be in accord with those of the writer, namely, that the biting depends more on the degree of the light than on the time of the day.

*The inoculation of bulls with the bodies of infected mosquitos.*

For purposes of inoculation most workers crushed their insects in some sort of liquid. Olitsky[3] and his collaborators prepared their lice for injection by crushing them in salt solution placed into sterile mortar, until a homogeneous emulsion was obtained. Curasson (*ante*) also crushed his insects and ticks in salt solution, whilst Noguchi crushed his mosquitos in Ringer's solution and applied the emulsion

[1] The references in this paragraph have been compiled from Howard, Dyar and Knab's *The Mosquitoes of North and Central America and the West Indies*, Vol. I (1912).

[2] This view of the French Commission has, however, been subsequently refuted by more than one worker.

[3] *Journ. Infect. Dis.*, XIX, p. 826 (1916).

on the scarified surface of the skin of guinea-pigs. Most other workers crushed their insects in salt solution. As it was found that dilution considerably interfered with the virulence of the rinderpest virus, the mosquitos in the present series of experiments were always inoculated into the animals under dry conditions. At first, whole uncrushed mosquitos were inserted, under sterile precautions, into incisions cut into the skin of susceptible bulls, the incisions being afterwards stitched up. Later on, crushing being considered necessary to facilitate the emergence of any ingested parasites, the mosquitos were crushed dry on sterilized knife-blades, prior to inoculation.[1] The factors that tend to vitiate the results of such inoculation experiments are, however, several. For example, as suggested by the Director of the Imperial Institute of Veterinary Research, there might be pyogenic organisms present, and the parasites might be phagocyted at the site of injection.

*The dissection of the internal parts of infected mosquitos and their examination for spirochætes.*

Noguchi does not appear to have examined the internal parts of the infected mosquitos for *Leptospira* (although he examined crushed mosquitos), and he seems to base his conclusions on circumstantial evidence when he writes, "The comparatively aseptic body cavity of the Stegomyia furnishes a secure shelter for the parasite, which undoubtedly penetrates the zone of safety as soon as it is taken into the stomach of the insect. Unlike many other parasites this organism is capable of penetrating the intact skin or bacteria-proof filter and hence it is probably an easy matter for it to pierce the tissue of the visceral organs" (*loc. cit.*, p. 410).

As in the present series of experiments none of the mosquitos became infective, it appeared reasonable to assume that if any parasites were ingested, they did not find their way into the hæmocœlic fluid and invade the salivary glands, or even if they did, they were considerably attenuated. In view of these considerations and also of the findings of previous workers, and particularly in view of the fact that the mosquitos were dissected shortly after their infective feeds, only the alimentary tract was especially examined for the parasites.

In the case of the few microscopic examinations that were made of the gut-contents of *A.*(*S.*) *albopicta*, the specimen was first killed with chloroform and, after its wings and legs had been snipped off, the specimen was placed in a drop of normal saline and its intestines were pulled out through the penultimate segment, by applying as slight traction as possible to avoid injury to the gut. The thin gut-wall was next carefully punctured with the point of a fine sterile pipette, which also drew in a minute quantity of the contents and these were now placed on a slide and quickly covered over with a cover-slip, the edges of the cover-slip being vase-

[1] These operations were carried out by the Director of the Imperial Institute of Veterinary Research.

lined to prevent evaporation. The preparation was then examined by the dark-ground illumination method.[1]

## II. Schedule of experiments.

### *The feeding experiments on bulls.*

*N.B.* The following observations have been transcribed from the notes as originally recorded.

In view of the supposed existence of a correlation between the biting propensities of *Stegomyia* and the degree of illumination, the condition of the weather has been indicated whenever this has been noted. "Light changing" means that there was an alternation of light and shade owing to uncertain weather while the feeding work was being carried on.

### *Mosquito 1.*

27-V. Found emerged. Enclosed in a cloth cage with several other males and females.

28-V. Removed from cage and enclosed in tube. Tried on Control 2076; did not bite. Fed on sugar solution.

29-V, 11 A.M. Tried on Control 2076; did not bite. Introduced 2 males into the tube.

30-V, 9 A.M. Tried on Control 2080; bit within 4 minutes of application. Males found dead.

31-V. Mosquito happy.

1-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.
3 P.M. Fed on sugar solution.

2-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.

3-VI, 9-30 A.M. Tried on Bull 2224 in chupper 53; did not bite. Mosquito very sluggish.
5-30 P.M. Fed on sugar solution.

4-VI, 6 P.M. Fed on sugar solution.

5-VI, 9-15 A.M. Tried on Bull 2223 in chupper 54; did not bite.

7-VI, 10 A.M. Tried on Bull 2245 in chupper 51; did not bite.

9-VI, 10-15 A.M. Tried on Bull 2241 in chupper 22; bit.

11-VI, 10 A.M. Tried on Bull 2243 in chupper 24; did not bite.

13-VI, 10 A.M. Tried on Bull 2240 in chupper 26; did not bite.

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[1] The writer desires to acknowledge his indebtedness to J. T. Edwards, Esq., Director of the Imperial Institute of Veterinary Research, for teaching him the technique of preparing such slides and the method of examining them dark-ground.

15-VI, 10-30 A.M. Tried for a very long time on Bull 2246 in chupper 29 ; did not bite.
17-VI, 9-30 A.M. Tried for a long time on Bull 2242 in chupper 30 ; did not bite.
19-VI, 10-30 A.M. Tried on Bull 2244 in chupper 32 ; did not bite. Mosquito moribund.
20-VI, 6 P.M. Mosquito moribund.
21-VI. Mosquito dead.

*Mosquito 2.*

31-V, 10 A.M. Found emerged. Tried on Control 2082 ; did not bite.
11 A.M. Fed on sugar solution.
1-VI, noon. Tried on Control 2079 ; did not bite.
3 P.M. Tried again on Control 2079 ; succeeded, after repeated attempts, in making it bite.
3-VI, 10 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
5-30 P.M. Fed on sugar solution.
4-VI, 6 P.M. Fed on sugar solution.
5-VI, 9-15 A.M. Tried on Bull 2224 in chupper 53 ; bit within 2 minutes of application. No feed of sugar solution to-day.
7-VI, 10 A.M. Tried on Bull 2223 in chupper 54 ; did not bite.
8-VI, 6 P.M. Offered sugar solution, but it did not feed.
9-VI, 10 A.M. Tried on Bull 2245 in chupper 51 ; bit.
11-VI, 10 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
13-VI, 10-30 A.M. Tried on Bull 2243 in chupper 24 ; succeeded, after repeated efforts, in making it bite. It took a very full feed.
15-VI, 10-15 A.M. Mosquito moribund.
6 P.M. Mosquito slightly revived and sucked a little of sugar solution.
16-VI, 6 P.M. Mosquito dead.

*Mosquito 3.*

31-V, 10 A.M. Found emerged. Tried on Control 2082 ; did not bite. Fed on sugar solution.
3 P.M. Mosquito escaped through netting.

*Mosquito 4.*

31-V, 10 A.M. Found emerged. Tried on Control 2079 at noon ; did not bite.
1-VI, 3-30 P.M. Fed on sugar solution.
2-VI, 3 P.M. Tried on Control 2085 ; did not bite. Mosquito moribund.

*Mosquito 5.*

30-V. Found emerged. Enclosed with 5 more females and 2 males in a bottle, which was kept inverted over its lid, a quantity of sugar solution and a few sheets of filter paper being placed in the cavity of the lid.

31-V, 10 A.M. Tried on Control 2082; did not bite. Sugar solution substituted by plain water.

noon. Tried again on Control 2082; did not bite.

3 P.M. Tried again on Control 2082; 3 engorged, of which one was enclosed in tube as No. 5, another as No. 6, while the third effected its escape through the netting, the unfed ones (3 females and one male) being enclosed together in bottle as No. 7.

2-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.

3-VI, 5-30 P.M. Fed on sugar solution.

4-VI, 10 A.M. Tried on Bull 2224 in chupper 53; did not bite.

6 P.M. Fed on sugar solution.

6-VI, 10-30 A.M. Tried on Bull 2223 in chupper 54; succeeded, after repeated attempts, in making it bite. Was not fed on sugar solution to-day.

8-VI, 10-15 A.M. Tried on Bull 2245 in chupper 51; did not bite. Offered sugar solution, but it did not feed.

10-VI, 11 A.M. Tried on Bull 2241 in chupper 22; did not bite.

12-VI, noon. Tried for a long time on Bull 2243 in chupper 24; did not bite.

14-VI, 11 A.M. Tried repeatedly on Bull 2240 in chupper 26; did not bite.

16-VI, 9-30 A.M. Tried repeatedly on Bull 2246 in chupper 29; did not bite.

18-VI, 9-30 A.M. Tried repeatedly on Bull 2242 in chupper 30; bit.

20-VI, 11 A.M. Tried on Bull 2244 in chupper 32; bit fairly readily.

22-VI, noon. Tried on Bull 2348 in chupper 39; did not bite, although seemed attempting to puncture. Bull somewhat old.

24-VI. Not tried.

26-VI, 1-15 P.M. Tried on Bull 2349 in chupper 42 for a long time; did not bite, although made efforts to puncture. Bull old.

28-VI, 12-30 P.M. Tried on Bull 2351 in chupper 43; bit readily.

30-VI, 12-30 P.M. Tried on Bull 2347 in chupper 45; bit.

4-VII, 10 A.M. Tried for 1½ hours on Bull 2440 in chupper 52; bit, but fed slightly. Labium of mosquito bent up (probably owing to sugar solution sticking to it) and mosquito unable to straighten it. As a result of careful dipping in water the adherent sugar seemed to be washed away and the labium returned to its normal position. Now tried again repeatedly on Bull 2440; did not bite.

8-VII, 11-30 A.M. Tried for a long time on Bull 2442 in chupper 54; after considerable trouble succeeded in making it take a full feed.

11-VII, 9 A.M. Mosquito stuck up in sugar; brushed, washed and released it by means of a pin, but mosquito moribund.
12-VII, 7 A.M. Mosquito found dead with abdomen swollen.

*Mosquito 6.*

*N.B.* For early history, see under *Mosquito 5.*

31-V, 3 P.M. Tried on Control 2082; bit.
2-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.
3-VI, 6 P.M. Fed on sugar solution.
4-VI, 10 A.M. Tried on Bull 2224 in chupper 53; bit.
6 P.M. Fed on sugar solution.
6-VI, 10-30 A.M. Tried on Bull 2223 in chupper 54; bit. Was not fed on sugar solution.
8-VI, 10-30 A.M. Tried on Bull 2245 in chupper 51; bit within a few minutes of application, although abdomen distended with sugar solution. Was not fed on sugar solution.
10-VI, 11 A.M. Tried on Bull 2241 in chupper 22; bit.
12-VI, noon. Tried on Bull 2243 in chupper 24; succeeded, after repeated attempts, in making it take a full feed.
14-VI, 11-30 A.M. Tried on Bull 2240 in chupper 26; bit.
16-VI, 10 A.M. Tried on Bull 2246 in chupper 29; bit.
18-VI, 11 A.M. Tried on Bull 2242 in chupper 30; bit.
20-VI, 11 A.M. Tried on Bull 2244 in chupper 32; bit very readily.
22-VI, noon. Tried on Bull 2348 in chupper 39; did not bite, although seemed to make efforts to puncture. Bull old.
24-VI. Not tried.
26-VI, 1-15 P.M. Tried for a long time on Bull 2349 in chupper 42; did not bite.
28-VI, 12-30 P.M. Tried on Bull 2351 in chupper 43; did not bite, although it made efforts to puncture. Mosquito sluggish.
29-VI. Mosquito very sluggish.
30-VI. Mosquito somewhat crumpled up and wet with sticky sugar solution. Revived to a considerable extent after it was cleaned and dried.
12-30 P.M. Tried for a long time on Bull 2347 in chupper 45; did not bite. Mosquito very sluggish.
1-VII. Endeavoured to revive it, but mosquito moribund.
2-VII. Mosquito found dead.

*Mosquito 8.*

*N.B.* This was one of the mosquitos enclosed in bottle as No. 7 (see under *Mosquito 5*).

1-VI, noon. Tried No. 7 on Control 2079; did not bite.
3 P.M. Tried No. 7 again on Control 2079; did not bite. Fed on sugar solution.

2-VI, 10 A.M. Tried No. 7 on Control 2085 ; did not bite.
3 P.M. Tried No. 7 on Control 2085 ; one only bit and this was isolated and enclosed in tube as No. 8. One of the remaining two mosquitos escaped through netting and the other died.
4-VI, 10 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
6 P.M. Fed on sugar solution.
6-VI, 10 A.M. Tried repeatedly on Bull 2224 in chupper 53 ; did not bite.
8-VI, 9-30 A.M. Tried on Bull 2223 in chupper 54 ; did not bite. Mosquito sluggish.
6 P.M. Fed on sugar solution.
10-VI, 10-30 A.M. Tried on Bull 2245 in chupper 51 ; did not bite. Mosquito sluggish.
12-VI, 12-30 P.M. Tried on Bull 2241 in chupper 22 ; did not bite. Mosquito sluggish.
14-VI, 10-30 A.M. Tried on Bull 2243 in chupper 24 ; did not bite. Mosquito sluggish.
16-VI, 10-30 A.M. Tried on Bull 2240 in chupper 26 ; did not bite. Mosquito moribund.
18-VI, morning. Mosquito found almost dead.
Evening. Mosquito dead.

*Mosquito 9.*

2-VI, 10 A.M. Found emerged. Enclosed, with a large number of males, in bottle, with sugar solution.
3-VI, 10 A.M. Isolated with one male and enclosed in tube as No. 9. Tried on Control 2088 ; did not bite. Fed on sugar solution.
2 P.M. Tried on Control 2088 ; did not bite. Bull very hairy. Mosquito damaged and almost dead.

*Mosquito 10.*

3-VI, 3 P.M. Enclosed with one more female and 2 males in tube. Fed on sugar solution.
4-VI, noon. Tried on Control 2198 ; did not bite.
5-VI 11 A.M. Tried on Control 2198 ; did not bite. Mosquito sluggish and slightly damaged.
Noon. Fed on sugar solution.
3-30 P.M. Tried on Control 2198 ; bit, but did not take a full feed.
7-VI, 10 A.M. Tried on Bull 2222 in chupper 53 ; did not bite. Mosquito moribund although abdomen distended with sugar solution.
10-VI, 5 P.M. Mosquito dead.

*Mosquito 11.*

4-VI, 4-30 P.M. 3 females and 1 male (emerged to-day) enclosed in bottle, with sugar solution.

5-VI, 11 A.M. Tried on Control 2198. One was fully gorged but escaped through netting; another also (unfed) escaped through netting; the third did not bite.
6-VI, 1 P.M. Tried the remaining female on Control 2198; did not bite.
4 P.M. Tried on Control 2198; bit.
7-VI. Enclosed the above gorged mosquito in tube as No. 11.
Noon. Fed on sugar solution.
8-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.
10-VI, 10 A.M. Tried on Bull 2224 in chupper 53 did not bite.
12-VI, 11 A.M. Tried on Bull 2223 in chupper 54; bit.
14-VI, 10 A.M. Tried on Bull 2245 in chupper 51; did not bite.
16-VI, 12-30 P.M. Tried on Bull 2241 in chupper 22; did not bite
18-VI, 11-30 A.M. Tried on Bull 2243 in chupper 24; bit.
20-VI, 9-30 A.M. Tried on Bull 2240 in chupper 26; did not bite.
22-VI, 9-30 A.M. Tried on Bull 2246 in chupper 29; did not bite.
23-VI, 10 A.M. Mosquito moribund.

*Mosquito 12.*

4-VI, 4-30 P.M. Found emerged. Enclosed in bottle with sugar solution. Mosquito probably virgin.
5-VI, 11 A.M. Tried on Control 2198; did not bite.
6-VI, 1 P.M. Tried on Control 2198; did not bite.
4 P.M. Tried again on Control 2198; bit.
7-VI, noon. Fed. on sugar solution.
8-VI, 10 A.M. Tried on Bull 2222 in chupper 52; did not bite.
10-VI, 10 A.M. Tried on Bull 2224 in chupper 53; did not bite.
12-VI, 11 A.M. Tried on Bull 2223 in chupper 54; bit.
14-VI, 10 A.M. Tried on Bull 2245 in chupper 51; did not bite.
16-VI, 12-30 P.M. Tried on Bull 2241 in chupper 24; did not bite.
18-VI, 11-30 A.M. Tried on Bull 2243 in chupper 24; did not bite.
20-VI, 9-30 A.M. Tried on Bull 2240 in chupper 26; did not bite. Mosquito slightly damaged.
22-VI, 9-30 A.M. Tried on Bull 2246 in chupper 29; did not bite.
23-VI, 10 A.M. Mosquito found dead.

*No. 13.* (*Two mosquitos.*)

6-VI, 3-30 P.M. Found emerged. Enclosed with another female and 4 males, in bottle, with sugar solution.
8-VI, noon. Tried on Control 2229; two fed. These two enclosed in tube as No 13.
10-VI, 10 A.M. Tried on Bull 2222 in chupper 52; both bit.
12-VI, 10-30 A.M. Tried on Bull 2224 in chupper 53; one bit.

14-VI, 9-30 A.M. One dead. Tried the other on Bull 2223 in chupper 53 ; did not bite.
16-VI, 11-30 A.M. Tried on Bull 2245 in chupper 51 ; bit.
18-VI, 11-30 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
20-VI, 10 A.M. Tried on Bull 2243 in chupper 24 ; bit.
22-VI, 10 A.M. Tried on Bull 2240 in chupper 26 ; did not bite. Mosquito not quite happy.
24-VI. Not tried.
26-VI, 11-30 A.M. Tried on Bull 2242 in chupper 30 ; bit.
28-VI, 11-30 A.M. Tried on Bull 2244 in chupper 32 ; did not bite.
29-VI. Mosquito stuck up in sugar solution and moribund.

*Mosquito 14.*

7-VI, 3 P.M. Found emerged. Enclosed with two more females and 4 males, in bottle, with sugar solution.
9-VI, 11-30 A.M. Tried on Control 2118 ; none bit.
3-30 P.M. Tried on Control 2118 ; one bit. Enclosed this in tube as No. 14.
11-VI, 9-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
13-VI, 10 A.M. Tried on Bull 2224 in chupper 53 ; bit.
15-VI, 9-45 A.M. Tried on Bull 2223 in chupper 54 ; did not bite.
17-VI, 11 A.M. Tried on Bull 2245 in chupper 51 ; bit within a few minutes of application and had a very full feed.
19-VI, 11 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
21-VI, 10 A.M. Tried on Bull 2243 in chupper 24 ; bit.
23-VI, 10 A.M. Mosquito moribund.

*No. 14(a).* (*Five Mosquitos.*) [*Kept in bottle, with sugar solution in lid.*]

This batch of mosquitos was composed as follows :—

(1) 5-VI. 1 newly-emerged male, 3 males (emerged on 1. VI) and 2 newly-emerged females, enclosed in bottle, with sugar solution.
6-VI, 3-30 P.M. Sugar solution substituted by plain water.
8-VI, noon. Tried on Control 2229 ; none bit.
8-VI. The females mentioned above, along with 3 more females (age not noted). enclosed in bottle, with sugar solution.
8-VI, 4 P.M. Tried on Control 2229 ; none bit.
9-VI, 11 A.M. 1 female dead.
11-30 A.M. Tried on Control 2118 ; none bit.
3-40 P.M. Tried again on Control 2118 ; none bit.
10-VI (hour not noted). Tried on Control 2107 ; none bit.
(2) 8-VI, 4-15 P.M. Enclosed 6 newly-emerged females with sugar solution, in bottle.
9-VI. Not tried.

10-VI, 3 P.M. Tried on Control 2107 ; 4 bit. One of the remaining two mosquitos killed as a result of being pressed by netting on rim of bottle.

The five engorged mosquitos now kept together in bottle with sugar solution in cavity of lid and labelled as No. 14 (*a*).

12-VI, 10 A.M. Tried on Bull 2222 in chupper 52 ; none bit.

13-VI, evening. 1 found dead in sugar solution.

14-VI, morning. Another found dead and a third escaped, so that only 2 now left. 9-15 A.M. Tried on Bull 2224 in chupper 53 ; did not bite. Mosquitos difficult to control.

16-VI, 11-15 A.M. Tried on Bull 2223 in chupper 54 ; 1 bit.

18-VI, noon. Tried on Bull 2245 in chupper 51 ; 1 bit, although its abdomen distended with sugar solution ; the other, although its abdomen thin, did not bite.

20-VI, 10 A.M. Tried on Bull 2241 in chupper 22 ; bit, although its abdomen distended with sugar solution ; the other, although very thin, did not bite.

22-VI. Not tried.

24-VI. Not tried.

26-VI, 11 A.M. Tried on Bull 2246 chupper 29 ; did not bite.

28-VI, 1 P.M. Enclosed the two mosquitos separately in tubes. Tried on Bull 2242 in chupper 30 ; did not bite.

30-VI, 11 A.M. Tried on Bull 2244 in chupper 32 ; both bit.

2-VII, 11-45 A.M. Tried on Bull 2348 in chupper 39 ; both bit. Bull old and its skin very thick.

4-VII, 2 P.M. Tried on Bull 2346 in chupper 41 ; 1 bit, the other did not bite (it looked gravid and was very sluggish, almost moribund).

6-VII, 1 P.M. Tried on Bull 2349 in chupper 42 ; did not bite. 1 mosquito only now alive.

8-VII, noon. Tried on Bull 2462(?) in chupper 43 for a long time ; took a full feed.

10-VII, 11-30 A.M. Tried for a long time on Bull 2347 in chupper 45 ; bit, but did not take a full feed. Mosquito sluggish and seems gravid.

14-VII, 10-50 A.M. Tried on Bull 2440 in chupper 52 ; bit, although mosquito much mutilated and incapable of locomotion, two of its legs being torn off and wings stuck together with sugar solution.

17-VII, 6 P.M. Mosquito moribund.

18-VII, 10 A.M. Mosquito found dead.

*Mosquito 15.* (*Mosquito not well-developed.*)

[Continued from (1) of the preceding experiment.]

11-VI, 11-30 A.M. Tried the remaining 3 mosquitos on Control 2126 ; did not bite. 3 P.M. Tried again on Control 2126 ; 1 bit. This now enclosed in tube as No. 15.

13-VI, 9-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite. Mosquito sluggish.
15-VI, 9-30 A.M. Tried on Bull 2224 in chupper 53 ; did not bite.
17-VI, 10-30 A.M. Tried on Bull 2223 in chupper 54 ; did not bite.
19-VI, 10 A.M. Tried on Bull 2245 in chupper 51 ; did not bite.
21-VI, 9-30 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
23-VI, 9 A.M. Tried on Bull 2243 in chupper 24 ; did not bite. Mosquito moribund.
11 A.M. Mosquito found dead.

*No. 15 (a) (Ten mosquitos.)*

9-VI, 3-30 P.M. Enclosed a large number of newly-emerged males and females in bottle, with sugar solution.
10 VI, noon. Enclosed a large number of females and only 1 male, in bottle with sugar solution.
12-VI, 3 P.M. All the above mosquitos enclosed together in a bottle. The mosquitos appeared to be somewhat unusually well-developed. Tried on Control 2126 ; 10 bit. The engorged females enclosed together in bottle as No. 15 (*a*).
14-VI, Not tried.
16-VI, 11 A.M. Tried on Bull 2224 in chupper 53 ; none bit.
17 VI, 5-30 P.M. A batch of eggs found laid on sugar solution.
18-VI, 12-30 P.M. Tried on Bull 2223 in chupper 54 ; none bit. A few more eggs found laid on sugar solution.
20-VI, 11-7 A.M. Tried on Bull 2245 in chupper 51 ; none bit. Extremely difficult to control the mosquitos.
22-VI, 10-15 A.M. Tried on Bull 2241 in chupper 22 ; none bit. 1 mosquito dead.
24-VI, Not Tried.
26-VI, 11-45 A.M. Tried on Bull 2240 in chupper 26 ; none bit. Only 5 mosquitos survive.
28-VI, 1-10 P.M. Tried on Bull 2246 in chupper 29 ; none bit. Only 2 mosquitos left and these kept separately in tubes.
30-VI, noon. Tried on Bull 2242 in chupper 30 ; did not bite. Only 1 mosquito left.
2-VII, 10 A.M. The surviving mosquito found dead.

*Mosquito 16.*

16-VI, 4-45 P.M. Found emerged. Fed on sugar solution and enclosed in tube.
17-VI, Not tried.
18-VI, 3-15 P.M. Tried on Control 2117 ; bit.
20-VI, 11-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
22 VI, 11 A.M. Tried on Bull 2224 in chupper 53; bit.

24-VI. Not tried.
26-VI, noon. Tried on Bull 2245 in chupper 51 ; bit.
28-VI 10-30 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
30-VI 11-30 A.M. Tried for a long time on Bull 2243 in chupper 24 ; did not bit
2-VII, 12-30 P.M. Tried on Bull 2240 in chupper 26 ; bit.
4-VII, 1 P.M. Tried on Bull 2246 in chupper 29 ; did not bite.
6-VII, 10-30 A.M. Tried for a long time on Bull 2242 in chupper 30 ; (?) slightly fed. Mosquito not happy and looks gravid.
8-VII, 11 A.M. Tried for a long time on Bull 2214 in chupper 32 ; showed no inclination to bite, rather repelled.
10-VII, noon. Tried on Bull 2348 in chupper 39 for a long time ; did not bite Mosquito mutilated and incapable of locomotion ; probably moribund
11-VII. Mosquito found dead.

*No. 17.* (*Two mosquitos.*)

15-VI, 3 P.M. Enclosed 2 newly-emerged mosquitos in tube. Fed on sugar solution.
16-VI, 3 P.M. Tried on Control 2086 ; none bit. Fed on sugar solution.
17-VI, Not tried. Fed on sugar solution.
18-VI, 3 P.M. Tried on Control 2117 ; 1 bit ; the other refused to bite, but as it made attempts to puncture when tried on my own arm about ten minutes later, it was again tried on Control 2117, when it bit readily.
20-VI, 11-30 A.M. Tried on Bull 2222 in chupper 52 ; none bit.
22-VI, 11 A.M. Tried on Bull 2224 in chupper 53 ; 1 bit.
24-VI, Not tried.
26-VI, noon. One dead. Tried the remaining one on Bull 2245 in chupper 51 ; bit.
28-VI, 10-30 A.M. Tried on Bull 2241 in chupper 22 ; did not bite.
30-VI 11-30 A.M. Tried on Bull 2243 in chupper 24 ; bit.
2-VII, 12-30 P.M. Tried for a long time on Bull 2240 in chupper 26 ; did not bite.
4-VII, 1 P.M. Tried on Bull 2246 in chupper 29 ; did not bite. Mosquito suddenly collapsed, when seemed about to bite. Applied moisture but it did not revive.

*Mosquito 18.*

14-VI. 3 newly-emerged females and 1 newly-emerged male of *A.*(*S.*) *thomsoni* and 1 newly-emerged female of *A.*(*S.*) *albopicta* fed on sugar solution and enclosed in tube.
15-VI, morning and afternoon. Tried on Control 2086 ; none bit. Transferred mosquitos to bottle.
16-VI, 3-30 P.M. Tried on Control 2086 ; none bit. Fed on sugar solution.
17-VI, noon. Tried on Control 2086 ; none bit. Fed on sugar solution.
18-VI, 3-30 P.M. Tried on Control 2117 ; only *albopicta* bit. Enclosed this in tube as No. 18.
20-VI, 11-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.

22-VI, 11 A.M. Tried on Bull 2224 in chupper 53 ; did not bite.
24-VI, Not tried.
26-VI, noon. Tried on Bull 2245 in chupper 51 ; bit.
28-VI, 10-30 A.M. Mosquito found dead.

*Mosquito 19.*

20-VI, 1 P.M. 3 newly-emerged females and 1 male fed on sugar solution and enclosed in tube.
Tried on Control 2130 ; did not feed, although made attempts to puncture.
3-30 P.M. Tried again on Control 2130 ; none bit.
21-VI, 11-45 A.M. Tried on Control 2078 ; 1 bit. Enclosed this separately in tube as No. 19.
23-VI, 9-30 A.M. Tried on Bull 2222 in chupper 52 ; bit.
25-VI. Not tried.
27-VI, 10-15 A.M. Tried on Bull 2223 in chupper 54 ; bit.
29-VI, 10-15 A.M. Tried on Bull 2245 in chupper 51 ; did not bite. Mosquito not quite happy.
1-VII, noon. Mosquito found dead.

*Mosquito 20.*

21-VI, 4 P.M. The two remaining mosquitos, which could not be made to bite Control at 11-45 A.M. (*vide* last experiment), were tried again on Control 2078 ; none bit. Fed on sugar solution.
22-VI, 3 P.M. Tried on Control 2078 ; none bit.
23-VI, 11 A.M. Tried on Control 2119 ; none bit.
26-VI, 4 P.M. Tried on Control 2066 ; 1 bit. Enclosed this separately in tube as No. 20.
28-VI, 11-45 A.M. Tried on Bull 2222 in chupper 52 ; did not bite. Mosquito not happy.
30-VI. Mosquito found dead.

*Mosquitos 21, 22, 23, 25 and 26.*

20-VI, 1 P.M. Enclosed a large number of females (emerged yesterday and to-day) in bottle, with sugar solution.
22-VI, 11 A.M. Tried on Control 2119 ; none bit. Offered fresh sugar solution.
26-VI, 4 P.M. Tried on Control 2066 ; none bit.
27-VI, noon. One of the females enclosed, together with a male, in a tube ; the rest enclosed singly in tubes.
3 P.M. Tried on Control 2119 ; 3 bit, including the one that had been enclosed with a male, but this fed only slightly. The three fed mosquitos enclosed singly in tubes as Nos. 21, 22 and 23.
28-VI, 4 P.M Tried the remaining on Control 2312 , 3 bit. 2 enclosed together in one tube as No. 25 ; the other enclosed singly in tube as No. 26.

*Mosquito 21.*

*N.B.* For early history see above.

27-VI, 3 P.M. Fed on Control 2119.
29-VI, 11 A.M. Tried on Bull 2222 in chupper 52 ; bit.
1-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit.
3-VII, 11 A.M. Tried on Bull 2223 in chupper 51 (removed from chupper 54) ; bit.
5-VII, 10-30 A.M. Tried on Bull 2245 in chupper 51 ; bit.
7-VII. 11 A.M. Tried on Bull 2241 in chupper 24 (removed from chupper 22) ; bit.
9-VII, 10-30 A.M. Tried for a long time on Bull 2243 in chupper 24 ; bit.
11-VII, 11 A.M. Tried for a long time on Bull 2240 in chupper 26 ; did not bite.
4 P.M. Tried again on Bull 2240 in chupper 26 ; did not bite.
13-VII, 11 A.M. Tried on Bull 2246 in chupper 29 ; did not bite.
15-VII. Mosquito found dead.

*Mosquito 22.*

*N.B.* For previous history see above.

27-VI, 4 P.M. Fed on Control 2119.
29-VI, 11 A.M. Tried on Bull 2222 in chupper 52 ; bit.
1-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit.
3-VII, 11 A.M. Tried on Bull 2223 in chupper 51 (removed from chupper 54) ; bit.
5-VII, 10-30 A.M. Tried for a long time on Bull 2245 in chupper 51 ; bit.
7-VII, 11 A.M. Tried for a long time on Bull 2241 in chupper 24 (removed from chupper 22) ; did not bite.
9-VII, 10-30 A.M. Tried on Bull 2243 in chupper 24 ; bit very readily
11-VII, 9 A.M. mosquito almost dead after ovipositing on muslin cover or tube. Tried to revive it but failed.

*Mosquito 23.*

*N.B.* For previous history see above.

27-VI, 4 P.M. Fed on Control 2119.
29-VI, 11 A.M. Tried on Bull 2222 in chupper 52 ; bit.
1-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit.
3-VII, 11 A.M. Tried on Bull 2223 in chupper 51 (removed from chupper 51) ; at first it went on moving about on muslin making occasional attempts at puncturing, but after a while it settled down all on a sudden and took a full feed.
5-VII. Mosquito found dead and distended.

*Mosquito 24.*

28-VI, 4 P.M. Fed slightly on Control 2312.
30-VI, 10-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
2-VII, 11 A.M. Tried on Bull 2224 in chupper 53 ; bit.
4-VII, noon. Tried on Bull 2223 in chupper 51 (removed from chupper 54) ; bit.
6-VII, noon. Tried for a fairly long time on Bull 2245 in chupper 51 ; bit.
8-VII, 10 A.M. Tried on Bull 2241 in chupper 24 (removed from chupper 22) ; did not bite. Mosquito sluggish.
10-VII, 10-30 A.M. Tried on Bull 2243 in chupper 24 ; bit, but not readily.
12-VII, 10-30 A.M. Tried on Bull 2240 in chupper 26 ; succeeded, with the greatest difficulty in making it bite. Mosquito somewhat sticky with sugar and very sluggish.
14-VII, 11-30 A.M. Tried on Bull 2246 in chupper 29 ; did not bite. Mosquito moribund, but appeared to revive a little when moisture was applied.
15-VII. Mosquito found dead.

*No. 25.* (*Two mosquitos.*)

*N.B.* For previous history see above.

28-VI, 4 P.M. Fed on Control 2312.
30-VI, 10-30 A.M. Tried on Bull 2222 in chupper 52 ; did not bite.
2-VII, 11 A.M. Tried on Bull 2224 in chupper 53 ; bit.
4-VII, noon. Tried on Bull 2223 in chupper 51 (removed from chupper 54) ; both bit.
6-VII. Both mosquitos found dead.

*Mosquito 26.*

*N.B.* For previous history see above.

28-VI, 4 P.M. Fed on Control 2312.
30-VI. Not tried.
2-VII, 11 A.M. Found dead.

*Mosquito 27.*

10-VII. Found emerged. Fed on sugar solution.
11-VII (hour not noted). Tried on Control 2323 ; did not bite.
12-VII, noon. Tried on Control 2340 ; bit.
14-VII, 10-30 A.M. Tried on Bull 2222 in chupper 52 ; bit.
16-VII, 11-30 A.M. Tried thrice at short intervals on Bull 2224 in chupper 53 ; it appeared unable to puncture the skin, although it seemed anxious to bite, but it bit when muslin cover of tube was pressed hard on ear to effect contact of mosquito with skin.

18-VII, 12-30 P.M.—2 P.M. Tried for about 1½ hours on Bull 2223 in chupper 51 ; did not bite although arduous efforts were made to make it do so.
20-VII, morning. Mosquito found dead.

*Mosquito 28.*

11-VII. Found emerged. Fed on sugar solution.
12-VII, 1 P.M. Tried on Control 2340 ; bit.
14-VII, 10-30 A.M. Tried on Bull 2222 in chupper 52 ; bit.
16-VII, 11-30 A.M. Abdomen of mosquito distended with a whitish mess. Tried repeatedly on Bull 2224 in chupper 53 ; did not feed although it appeared to puncture, but bit when muslin was stretched.
18-VII, 12-30 P.M. Tried repeatedly on Bull 2223 in chupper 51 ; bit. It fed in two instalments. The excrement voided immediately after the first feed appeared to consist of blood, whilst that passed after the second feed was, as is usual, *liquor sanguinis*.
20-VII, noon. Mosquito quite happy and active. Tried repeatedly and at intervals on Bull 2245 in chupper 51 ; bit.
22-VII, noon. Tried on Bull 2241 in chupper 24 ; bit fairly readily.
24-VII, 7-30 A.M. Mosquito found dead and distended.

*Mosquito 29.*

11-VII. Found emerged.
3 P.M. Fed on sugar solution.
12-VII, noon. Tried on Control 2340 ; did not bite.
4-30 P.M. Tried on Control 2340 ; did not bite.
13-VII, noon. Tried on Control 2340 ; bit.
15-VII, 10-30 A.M. Tried on Bull 2222 in chupper 53 (removed from chupper 52 ; bit.
17-VII, 11 A.M. Mosquito found dead.

*Mosquito 30.*

12-VII. Found emerged.
3-30 P.M. Fed on sugar solution.
13-VII, noon. Tried on Control 2340 ; bit.
15-VII, 10-30 A.M. Tried on Bull 2222 in chupper 53 (transferred from chupper 52) ; bit readily.
17-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit readily.
19-VII, 11-20 A.M. Tried repeatedly on Bull 2223 in chupper 51 ; bit. Noticed that muslin must be kept sufficiently stretched in order to secure a successful feed ; in the present case muslin had to be removed and retied.
21-VII, noon. Tried on Bull 2245 in chupper 51 ; bit readily.
23-VII, 10-30 A.M. Mosquito found dead.

*Mosquito 31.*

12-VII, 3-30 P.M. Found emerged. Fed on sugar solution.
13-VII, noon. Tried on Control 2340 ; bit.
15-VII, 10-30 A.M. Tried on Bull 2222 in chupper 53 (transferred from chupper 52) ; bit readily.
17-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit readily.
19-VII, 11-30 A.M. Tried repeatedly and for a long time on Bull 2223 in chupper 51 ; bit. Noticed that muslin must be kept stretched to effect a good feed.
21-VII, noon. Mosquito found dead, although abdomen still distended with blood.

*Mosquito 32.*

12-VII. Found emerged.
3-30 P.M. Fed on sugar solution.
13-VII, noon. Tried on Control 2340; bit.
15-VII, 10-30 A.M. Tried on Bull 2222 in chupper 53 , bit readily.
17-VII, 10-30 A.M. Tried on Bull 2224 in chupper 53 ; bit readily.
19-VII, noon. Mosquito sluggish. Tried repeatedly on Bull 2223 in chupper 51 ; did not bite.
5 P.M. Tried repeatedly on Bull 2223 in chupper 51 ; did not bite. Mosquito damaged as a result of continuous handling.
20-VII, 10 A.M. Mosquito found dead.

*Mosquito 33.*

12-VII Found emerged.
3-30 P.M. Fed on sugar solution.
13-VII, 12-30 P.M. Tried on Control 2340 ; did not bite.
14-VII, 11 A.M.—2 P.M. Tried on Control 2475 (young bull) ; bit.
16-VII, 1 P.M. Tried for about 2 hours on Bull 2222 in chupper 53 ; did not bite. Proboscis bent upwards and mosquito never attempted to puncture.
18-VII, 10-30 A.M. Tried repeatedly on Bull 2224 in chupper 53 ; bit.
20-VII, 10-30 A.M. Mosquito found dead.

*Mosquito 34.*

12-VII, 3-30 P.M. Found emerged. Fed on sugar solution.
13-VII, noon. Tried on Control 2340 ; did not bite.
14-VII, 11 A.M.—2 P.M. Tried on Control 2475 ; bit.
16-VII, 11 A.M.—1 P.M. Tried repeatedly and at intervals on Bull 2222 in chupper 53 ; bit. Bull difficult to immobilize.
18-VII, 10-30 A.M.—12-30 P.M. Tried at intervals on Bull 2224 in chupper 53 ; bit, but was not fully gorged.

20-VII, 11-30 A.M. Tried at intervals and for a long time on Bull 2223 in chupper 53 ; bit, but was not fully gorged. Mosquito very sluggish.
21-VII, 8 A.M. Mosquito found dead.

*Mosquito 35.*

13-VII, noon. Found emerged.
4 P.M. Fed on sugar solution.
14-VII, 11 A.M.—1 P.M. Tried on Control 2475 ; bit.
16-VII, 11 A.M.—1 P.M. Tried on Bull 2222 in chupper 53 ; bit very readily. Bull extremely difficult to immobilize.
18-VII, 10-30 A.M. Tried at intervals for about 1½ hours on Bull 2224 in chupper 53 ; bit. Abdomen of mosquito distended with a white mass.
20-VII, 11 A.M. Tried on Bull 2223 in chupper 51 ; bit readily.
22-VII, 11-30 A.M. Tried on Bull 2245 in chupper 51 ; bit fairly readily.
24-VII, 10-40 A.M. Tried repeatedly on Bull 2241 in chupper 24 ; bit. Abdomen of mosquito distended with a white mass.
26-VII, 11 A.M. Tried repeatedly on Bull 2243 in chupper 24 ; did not bite.
28-VII, 10-45 A.M. Tried on Bull 2240 in chupper 26 ; did not bite.
30-VII, 3-25 P.M. Tried on Bull 2246 in chupper 29 ; bit fairly readily.
1-VIII, 11-30 A.M. Tried on Bull 2242 in chupper 30 ; bit fairly readily. Abdomen distended with a white mass.
3-VIII, 11-30 A.M. Tried on Bull 2224 in chupper 26 (transferred from chupper 32) ; did not bite. Mosquito sluggish.
5-VIII, 11-30 A.M. Tried on Bull 2348 in chupper 39 ; did not bite.
7-VIII, 11-30 A.M. Tried on Bull 2626 in chupper 41 ; bit.
9-VIII, 11 A.M. Tried on Bull 2599 in chupper 42 ; did not bite. Except for the anterior end, which was bordered with a black material, the whole abdomen was distended with a white mass.
11-VIII, 11 A.M. Tried on Bull 2462(?) in chupper 43 ; did not bite, although it made attempts to puncture. Tried on inner sides of ear of the animal, but with no better success. Bull old, its skin thick and hairy.
13-VIII, 5-45 P.M. Mosquito crushed and inoculated into Bull 2723 (*vide* Inoculation experiments.)

*Mosquito 36.*

11-VII, Found emerged.
3 P.M. Fed on sugar solution.
12-VII, noon. Tried on Control 2340 ; did not bite. Bull old.
4 P.M. Tried on Control 2340 ; did not bite.
13-VII, noon. Tried on Control 2340 ; did not bite.
14-VII, 11 A.M.—1 P.M. Tried on Control 2475 (young bull) ; did not bite.
15-VII, 12-30 P.M. Tried on Control 2472 ; bit.

17-VII, 11-30 A.M. Tried on Bull 2222 in chupper 53 ; bit readily.
19-VII, 12-30 P.M. Tried repeatedly on Bull 2224 in chupper 53 ; fed in two instalments. Bit only when muslin was properly stretched.
21-VII, 10-30 A.M. Tried repeatedly on Bull 2223 in chupper 51 ; bit.
23-VII, 12-30 P.M. Tried repeatedly and at intervals on Bull 2245 in chupper 51 ; did not bite.
25-VII, 11 A.M. Tried at intervals on Bull 2241 in chupper 24 ; did not bite.
27-VII. Mosquito found dead.

*Mosquito 37.*

13-VII, noon. Found emerged.
4 P.M. Fed on sugar solution.
14-VII, 11 A.M.—1 P.M. Tried on Control 2475 (young bull) ; did not bite.
15-VII, 12-30 P.M. Tried on Control 2472 ; bit.
17-VII, 11-30 A.M. Tried on Bull 2222 in chupper 53 ; bit fairly readily.
19-VII, 12-45 P.M. Tried repeatedly on Bull 2224 in chupper 53 ; fed in two instalments. Bit at the last moment, when it was going to be given up as hopeless.
21-VII, 10-30 A.M.—12 noon. Tried repeatedly on Bull 2223 in chupper 51 ; bit when it was going to be given up as hopeless.
23-VII, 12-30 P.M. Tried repeatedly and at intervals on Bull 2245 in chupper 51 ; did not bite.
25-VII, 11 A.M. Tried at intervals on Bull 2241 in chupper 24 ; did not bite.
27-VII, 10-30 A.M. Tried on Bull 2243 in chupper 24 ; did not bite.
29-VII, 10-30 A.M. Tried for about 1½ hours on Bull 2240 in chupper 26 ; did not bite.
31-VII, 10-30 A.M. Tried repeatedly on Bull 2246 in chupper 29 ; did not bite. Mosquito not fed on raisins or sugar since evening of 28-VII. Mosquito without one leg and appears somewhat frantic, although fairly active.
2-VIII, 10-30 A.M. Tried repeatedly on Bull 2242 in chupper 30 ; did not bite. Condition of mosquito same as on 31-VII.
noon. Put in raisins.
4-VIII, 10 A.M. Tried on Bull 2244 in chupper 26 ; did not bite.
6-VIII, 10-30 A.M. Tried on Bull 2627 (young bull) in chupper 39 ; did not bite.
8-VIII, 11 A.M. Tried for a long time on Bull 2626 in chupper 41 ; did not bite. Mosquito thin but fairly active.
10-VIII, morning. Mosquito moribund.
1 P.M. Tried on Bull 2599 in chupper 42 ; did not bite. Mosquito almost dead.

*Mosquito 38.*

13-VII. Found emerged. Tried on Control 2472 ; did not bite, although when placed on my arm it made efforts to puncture the skin.
3 P.M. Fed on sugar solution.

14-VII, 11 A.M.—1 P.M. Tried on Control 2475 (young bull); did not bite.
15-VII, 12-30 P.M. Tried on Control 2472; bit.
17-VII, 11-30 A.M. Tried on Bull 2222 in chupper 53; did not feed readily and fed only slightly. While refixing loose muslin, mosquito lost two legs and was, in other ways, much mutilated as it made frantic efforts to escape.

*Mosquito 39.*

14-VII, 11 A.M. Found emerged.
3 P.M. Fed on sugar solution.
15-VII, 12-30 P.M. Tried on Control 2472; did not bite.
Fed on sugar solution.
16-VII, 2-30 P.M. Tried on Control 2472; did not bite.
3-30 P.M. Fed on sugar solution.
17-VII, 1 P.M. Tried on Control 2404; bit.
19-VII, 1-20 P.M. Tried repeatedly on Bull 2222 in chupper 53; bit, but was not fully gorged.
21-VII, 12-45 P.M. Tried on Bull 2224 in chupper 53; bit fairly readily.
23-VII, 11 A.M. Tried repeatedly on Bull 2223 in chupper 51; bit.
24-VII, 6 P.M. Tied up raisin on muslin of tube.
25-VII, 7-30 A.M. Abdomen of mosquito full of juice of raisin.
Noon. Tried repeatedly and at intervals on Bull 2245 in chupper 51; did not bite.
27-VII, 1 P.M. Tried repeatedly and at intervals on Bull 2241 in chupper 24; did not bite.
29-VII, 12-30 P.M. Tried repeatedly and at intervals on Bull 2243 in chupper 24; did not bite.
31-VII, 11 A.M. Tried repeatedly and at intervals on Bull 2240 in chupper 26; did not bite. Mosquito all on a sudden collapsed while being tried (somewhat violently) on this bull. Bull difficult to immobilize. Mosquito had no raisin or sugar solution since evening of 28-VII.

*Mosquito 40.*

15-VII, noon. Found emerged.
1 P.M. Fed on sugar solution.
16-VII, 2-30 P.M. Tried on Control 2472; did not bite
3-30 P.M. Fed on sugar solution.
17-VII, 1 P.M. Tried on Control 2404; did not bite.
3 P.M. Tried repeatedly on Control 2404; bit.
19-VII, 1-30 P.M. Tried repeatedly on Bull 2222 in chupper 53; bit.
21-VII, 1 P.M. Tried at intervals on Bull 2224 in chupper 53; bit. Bull difficult to immobilize.

23-VII, 11-15 A.M. Tried repeatedly on Bull 2223 in chupper 51 ; bit.
24-VII, 6-P.M. Changed muslin and tied up fresh raisin.
25-VII, 7-30 A.M. Abdomen of mosquito distended with juice of raisin.
Noon. Tried repeatedly and at intervals on Bull 2245 in chupper 51 ; did not bite.
27-VII, noon. Tried repeatedly and at intervals on Bull 2241 in chupper 24 ; bit. Noticed that when there was an excess of moisture in the muslin, the mosquito refused to bite, and it bit when the moisture had been allowed to evaporate.
29-VII, 12-30 P.M. Tried repeatedly and at intervals on Bull 2243 in chupper 24 ; did not bite.
31-VII, 11 A.M. Tried repeatedly and at intervals on Bull 2240 in chupper 26 ; did not bite. No raisin given since evening of 28-VII.
2-VIII, 11-10 A.M. Tried repeatedly on Bull 2246 in chupper 29 ; bit.
4-VIII, 12-45 P.M. Tried on Bull 2242 in chupper 30 ; bit fairly readily.
6-VIII, 11-30 A.M. Tried on Bull 2244 in chupper 26 ; did not bite.
8-VIII, 11-45 A.M. Tried on Bull 2627 in chupper 39 ; did not bite.
10-VIII, 11-30 A.M. Mosquito found dead.

*Mosquito 41.*

15-VII, noon. Found emerged.
1 P.M. Fed on sugar solution.
16-VII, 2-30 P.M. Tried on Control 2472 ; did not bite.
3-30 P.M. Fed on sugar solution.
17-VII, 1 P.M. Tried on Control 2404 ; did dot bite.
1-10 P.M. Tried on my arm and it showed readiness to bite.
3 P.M. Tried again on Control 2404 ; bit.
19-VII, 1-45 P.M. Tried on Bull 2222 in chupper 53 ; bit readily and became fully gorged very quickly.
21-VII, 1-10 P.M. Tried at intervals on Bull 2224 in chupper 53 ; bit.
23-VII, 11-50 A.M. Tried repeatedly on Bull 2223 in chupper 51 ; did not bite.
Applied moisture on skin of animal to see if that would induce mosquito to bite, but result was negative.
24-VII, 6 P.M. Changed muslin and tied up fresh raisin.
25-VII, 7-30 P.M. Abdomen of mosquito full of juice of raisin.
noon. Tried repeatedly and at intervals on Bull 2245 in chupper 51 ; did not bite.
27-VII, 1 P.M. Tried repeatedly and at intervals on Bull 2241 in chupper 24 ; did not bite.
29-VII, 12-30 P.M. Tried repeatedly and at intervals on Bull 2243 in chupper 24 ; did not bite.

31-VII, 11 A.M. Tried repeatedly and at intervals on Bull 2240 in chupper 26; did not bite. No raisin given since evening of 28-VII.
2-VIII, 11-10 A.M. Mosquito found dead.

*Mosquito 42.*

20-VII, 4 P.M. Found emerged. Was not fed on sugar solution or raisin.
21-VII. No Control available to-day.
3-40 P.M. Fed on sugar solution.
22-VII, 2 P.M. Fed on sugar solution.
No Control available to-day.
23-VII, 3 P.M. Tried on Control 2436; bit.
24-VII, 6 P.M. Tied up raisin on muslin.
25-VII, 7-30 A.M. Abdomen of mosquito full of juice of raisin.
12-45 P.M. Tried repeatedly on Bull 2222 in chupper 53; did not bite. Abdomen of mosquito still full of raisin juice.
27-VII, 1-15 P.M. Tried on Bull 2224 in chupper 53; did not bite. Skin of animal wet, having been washed with water to remove adherent dung.
29-VII, 1 P.M. Tried repeatedly on Bull 2223 in chupper 51; did not bite.
31-VII, noon. Tried repeatedly on Bull 2245 in chupper 51; did not bite, although mosquito made attempts to puncture.
2-VIII, 1-45 P.M. Tried repeatedly on Bull 2241 in chupper 24; bit.
4-VIII, 11 A.M. Tried on Bull 2243 in chupper 24; did not bite.
6-VIII, 11-30 A.M. Tried on Bull 2240 in chupper 26; did not bite.
8-VIII, noon. Tried on Bull 2246 in chupper 29; fed slowly and was not fully gorged.
10-VIII, 11-30 A.M. Tried on Bull 2242 in chupper 30; bit.
12-VIII, 11 A.M. Tried on Bull 2244 in chupper 26; bit.
13-VIII, 5-45 P.M. Crushed and inoculated into Bull 2723. (*Vide* Inoculation experiments.)

*Mosquito 43.*

24-VII. Found emerged.
3-30 P.M. Enclosed in tube with raisin.
25-VII, 2-30 P.M.—4-30 P.M. Tried repeatedly on Control 2354 (young bull); did not bite.
26-VII, noon and 3 P.M. Tried repeatedly on Control 2436; did not bite.
4 P.M. Removed raisin.
27-VII, 3-30 P.M. Tried on Control 2350; bit.
29-VII, 1-27 P.M. Tried repeatedly and at intervals on Bull 2222 in chupper 53; mosquito succeeded in biting after some attempts. Mosquito has had no raisin since infective feed.

31-VII, 12-30 P.M. Tried repeatedly on Bull 2224 in chupper 53; mosquito succeeded in biting after some attempts.
2-VIII, 12-20 P.M. Tried repeatedly and at intervals on Bull 2223 in chupper 51; bit.
4-VIII, 11-30 A.M. Tried on Bull 2245 in chupper 51; did not bite.
6-VIII, noon. Tried on Bull 2241 in chupper 24; bit.
8-VIII, 11 A.M. Mosquito found dead.

*Mosquito 44.*

24-VII, Found emerged.
3-30 P.M. Enclosed in tube with raisin.
25-VII, 2-30—4-30 P.M. Tried repeatedly on Control 2354 (young bull); did not bite.
26-VII, noon and 3 P.M. Tried on Control 2436; did not bite.
4 P.M. Removed raisin.
27-VII, 3-30 P.M. Tried on Control 2350; bit.
29-VII, 1-15 P.M. Tried on Bull 2222 in chupper 53; bit. Mosquito kept without raisin since infective feed.
31-VII, 12-30 P.M. Tried on Bull 2224 in chupper 53; bit.
2-VIII, 12-30 P.M. Tried on Bull 2223 in chupper 51; bit readily.
4-VIII, 11-30 A.M. Tried on Bull 2245 in chupper 51; bit.
6-VIII, noon. Tried on Bull 2241 in chupper 24; bit.
8-VIII, noon. Tried on Bull 2243 in chupper 24; bit.
10 VIII, 11 A.M. Tried on bull 2240 in chupper 26; bit.
12-VIII, 11-30 A.M. Tried on Bull 2246 in chupper 29; bit readily.
13-VIII, 5-45 P.M. Crushed and inoculated into Bull 2723. (*Vide* Inoculation experiments.)

*Mosquito 45.*

23-VII. Found emerged.
28-VII, 12-30 P.M. Tried on Control 2410; bit.
29-VII, 5 P.M. Dissected and examined dark-ground.

*Mosquito 46.*

24-VII. Found emerged.
3-30 P.M. Enclosed with raisin.
25-VII, 12-30 P.M.—4-30 P.M. Tried repeatedly on Control 2354 (young bull); did not bite.
26-VII, noon and 3 P.M. Tried repeatedly on Control 2436; did not bite.
4 P.M. Removed raisin.
27-VII, 3-30 P.M. Tried on Control 2350; did not bite.
28-VII, 12-30 P.M. Tried on Control 2410; bit.

30-VII, 3 P.M. Tried on Bull 2222 in chupper 53 ; bit readily.
No raisin given since infective feed.
1-VIII, 11-10 A.M. Tried repeatedly on Bull 2224 in chupper 53 ; did not bite. Abdomen distended since last feed.
3-VIII. Not tried. Offered raisin.
5-VIII, 9 A.M. Removed raisin.
10-30 A.M. Tried on Bull 2245 in chupper 51 ; did not bite.
7-VIII, 10-30 A.M. Tried on Bull 2241 in chupper 24 ; did not bite.
9-VIII, 11-30 A.M. Tried on Bull 2243 in chupper 24 ; did not bite.
11-VIII, 3 P.M. Tried on Bull 2240 in chupper 26 ; did not bite.
13-VIII, 8 A.M. Mosquito found dead.

*Mosquito 47.*

24-VII. Found emerged.
3-30 P.M. Enclosed with raisin.
25-VII, 12-30—4-30 P.M. Tried repeatedly on Control 2354 (young bull) ; did not bite.
26-VII, noon and 3 P.M. Tried repeatedly on Control 2436 ; did not bite. Raisin removed.
27-VII, 3-30 P.M. Tried on Control 2350 ; did not bite.
28-VII, 12-30 P.M. Tried on Control 2410 ; did not bite.
29-VII, 3 P.M. Tried on Control 2350 ; bit. Removed raisin.
31-VII, 1 P.M. Tried on Bull 2222 in chupper 53 ; it seemed to bite, but it could not be definitely ascertained whether it had imbibed blood (abdomen already considerably distended). There was just a suggestion of blood in abdomen.
2-VIII, 2-45 P.M. Tried repeatedly on Bull 2224 in chupper 53 ; bit, but was not fully gorged.
4-VIII, noon. Tried on Bull 2223 in chupper 51 ; bit fairly readily and fed very greedily.
6-VIII, 1-30 P.M. Tried on Bull 2245 in chupper 51 ; did not bite.
8-VIII, 12-15 P.M. Tried on Bull 2241 in chupper 24 ; did not bite.
10-VIII, 10 A.M. Tried on Bull 2243 in chupper 24 ; bit.
12-VIII, 10-45 A.M. Tried on Bull 2240 in chupper 26 ; bit, but was not fully gorged.
13-VIII, 5-45 P.M. Crushed and inoculated into Bull 2723. (*Vide* Inoculation experiments.)

*Mosquito 48.*

27-VII. Found emerged.
4-30 P.M. Enclosed with raisin.
28-VII, 12-30 P.M. Tried on Control 2410 ; did not bite. Removed raisin.
29-VII. Not tried.

30-VII, 11-30 A.M. Tried repeatedly and at intervals on Control 2420; bit.
6-30 P.M. Dissected and examined dark-ground.

*Mosquito 49.*

28-VII, 1 P.M. Found emerged. Enclosed with raisin.
29-VII. Not tried. Raisin not removed.
30-VII, 11-30 A.M. Tried repeatedly and at intervals on Control 2420; bit.
1-VIII, 8 A.M. Removed raisin.
10-40 A.M. Tried on Bull 2222 in chupper 53; bit readily.
3-VIII, 11 A.M. Tried on Bull 2223 in chupper 51; bit. Mosquito escaped.

*Mosquito 50.*

27-VII, 4-30 P.M. Found emerged. Enclosed with raisin.
28-VII, 12-30 P.M. Tried on Control 2410; did not bite. Removed raisin.
29-VII. Not tried.
30-VII, 11-30 A.M. Tried repeatedly and at intervals on Control 2420; did not bite.
31-VII, 3-45 P.M. Tried on Control 2420; bit.
2-VIII, 1 P.M. Tried on Bull 2222 in chupper 53; bit fairly readily.
3-VIII, 4-45 P.M. Inoculated into Bull 2653. (*Vide* Inoculation experiments.)

*Mosquito 51.*

28-VII, 1 P.M. Found emerged. Enclosed with raisin.
29-VII, 3 P.M. Not tried.
30-VII, 11-30 A.M. Tried repeatedly and at intervals on Control 2420; did not bite.
31-VII, 3-45 P.M. Tried on Control 2420; did not bite. Raisin not yet removed.
1-VIII, noon. Tried on Control 2420; bit.
3-VIII, 4-15 P.M. Inoculated into Bull 2654. (*Vide* Inoculation experiments.)

*Mosquito 52.*

27-VII, 4-30 P.M. Found emerged. Enclosed with raisin.
28-VII, 12-30 P.M. Tried on Control 2410; did not bite. Removed raisin.
29-VII, Not tried.
30-VII, 11-30 A.M. Tried repeatedly and at intervals on Control 2420; did not bite.
31-VII, 3-45 P.M. Tried on Control 2420; did not bite.
1-VIII, noon. Tried repeatedly and at intervals on Control 2420; did not bite.
2-VIII, 3 P.M. Tried repeatedly on Control 2412; bit.
3-VIII, 4-22 P.M. Inoculated into Bull 2661. (*Vide* Inoculation experiments.)

*Mosquitos 53, 54 and 55.*

1-VIII, 2-30 P.M. Enclosed 3 newly-emerged females singly in tubes, with raisins.
2-VIII, 1-30 P.M. Tried on Control 2412; did not bite.
3-30 P.M. Removed raisins.

3-VIII, 3 P.M. Tried on Control 2432 ; did not bite.
4-VIII, 4 P.M. Tried on Control 2441 ; did not bite.
5-VIII, 1 P.M. Tried on Control 2411 ; did not bite.
2 P.M. All the three mosquitos enclosed together in bottle.
6-VIII, 3-30 P.M. Again enclosed the three mosquitos separately in tube.
4-30 P.M. Tried on Control 2441 ; none bit.
7-VIII, noon. Tried on Control 2434 ; all the three bit. Numbered these 53, 54 and 55.

*Mosquito 53.*

*N.B.* For previous history see above.

7-VIII, noon. Tried on Control 2434 ; bit.
9-VIII, 12-30 P.M. Tried on Bull 2222 in chupper 53 ; bit.
11-VIII, noon. Tried on Bull 2224 in chupper 53 ; bit fairly readily.
13-VIII, 11 A.M. Tried on Bull 2223 in chupper 51 ; did not bite.
6 P.M. Crushed and inoculated into Bull 2720. (*Vide* Inoculation experiments.)

*Mosquito 54.*

*N.B.* For previous history see above.

7-VIII, noon. Tried on Control 2434 ; bit.
9-VIII, 12-30 P.M. Tried on Bull 2222 in chupper 53 ; did not bite.
11-VIII, noon. Tried repeatedly and at intervals on Bull 2224 in chupper 53 ; did not bite.
13-VIII, 11 A.M. Tried repeatedly on Bull 2223 in chupper 51 ; bit.
6 P.M. Crushed and inoculated into Bull 2720. (*Vide* Inoculation experiments.)

*Mosquito 55.*

*N.B.* For previous history see above.

7-VIII, Noon. Tried on Control 2434 ; bit.
9-VIII, 12-30 P.M. Tried on Bull 2222 in chupper 53 ; did not bite.
11-VIII, noon. Tried on Bull 2224 in chupper 53 ; did not bite.
13-VIII, 11 A.M. Tried on Bull 2223 in chupper 51 ; did not bite.
6 P.M. Crushed and inoculated into Bull 2720. (*Vide* Inoculation, experiments.)

*Mosquitos 56 and 57.*

10-VIII 2 P.M. Found emerged. Enclosed two females and one male with raisins in a small bottle.
12-VIII, 12-30 P.M. Removed raisins.
14-VIII, noon. Tried on Control 2461 ; both females bit. The gorged females enclosed separately in tubes as 56 and 57.

*Mosquito 56.*

*N.B.* For previous history see above.

16-VIII, 2 P.M. Found dead.

*Mosquito 57.*

*N.B.* For previous history see above.

14-VIII, noon. Tried on Control 2461 ; bit. Enclosed with raisin.
16-VIII, 2 P.M. Tried on Bull 2224 in chupper 52 ; did not bite.
18-VIII, 10 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
4 P.M. Removed raisin.
20-VIII, 10 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
22-VIII, 10-30 A.M. Tried repeatedly on Bull 2224 in chupper 52 ; bit.
24-VIII, 10-30 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite.
26-VIII, 11 A.M. Tried on Bull 2242 in chupper 53 ; bit.
28-VIII, 11-30 A.M. Tried on Bull 2242 in chupper 53 ; did not bite. Abdomen of mosquito distended with a white mass.
30-VIII, 11 A.M. Tried on Bull 2242 in chupper 53 ; did not bite. Abdomen of mosquito still somewhat distended with a white mass.
1-IX, 4-30 P.M. Mosquito found dead.

*Mosquito 58.*

20-VIII, noon. Emerged. Enclosed with raisin.
21-VIII, noon. Tried on Control 2688 ; did not bite. Removed raisin.
22-VIII, 1 P.M. Tried repeatedly and at intervals on Control 2602 ; bit.
24-VIII, 10-30 A.M. Tried repeatedly on Bull 2224 in chupper 52 ; bit.
26-VIII, 10-30 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite.
28-VIII, 11 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite. Mosquito suddenly died.

*Mosquito 59.*

21-VIII, 3-45 P.M. Emerged. Enclosed with raisin.
22-VIII, 1 P.M. Tried on Control 2602 ; did not bite.
3 P.M. Removed raisin.
23-VIII, noon. Tried on Control 2612 ; bit, but was not fully gorged.
25-VIII, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; bit fairly readily, but was not fully gorged.
27-VIII, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
29-VIII, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; bit.
31-VIII, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; bit.

2-IX, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; bit, but was not fully gorged ; Mosquito busy brushing off moisture.
3-IX, evening. Mosquito moribund.
4-IX, morning. Mosquito found dead.

*Mosquito 60.*

19-VIII, noon. Found emerged.
4 P.M. Enclosed with raisin.
20-VIII, noon. Removed raisin.
21-VIII, noon. Tried on Control 2688 ; did not bite.
22-VIII, 1-2 P.M. Tried repeatedly and at intervals, for a long time, on Control 2602 ; did not bite.
2 P.M. Offered raisin again.
23-VIII, noon. Tried repeatedly and at intervals on Control 2612 ; did not bite. Removed raisin.
24-VIII, 1 P.M. Tried on Control 2612 ; bit.
26-VIII, 10-30 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite.
28-VIII, 11 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; bit, but was not fully gorged.
30-VIII, 10 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite.
1-IX, 4-30 P.M. Mosquito found dead.

*Mosquito 61.*

22-VIII, noon. Found emerged. Enclosed with raisin.
23-VIII, noon. Tried on Control 2612 ; did not bite. Removed raisin.
24-VIII, 1 P.M. Tried on Control 2612 ; bit, but was not fully gorged. Tried several times afterwards, but could not make it take a full feed.
26-VIII, 10-30 A.M. Tried on Bull 2224 in chupper 52 ; bit, but did not take a full feed.
28-VIII, 10 A.M. Tried on Bull 2224 in chupper 52 ; bit very readily.
30-VIII, 10 A.M. Tried repeatedly on Bull 2224 in chupper 52 ; did not bite.
1-IX, 4-30 P.M. Tried on Bull 2224 in chupper 52 ; bit.
3-IX, evening. Mosquito found dead.

*Mosquito 62.*

25-VIII, 12-45 P.M. Found emerged. Enclosed with raisin.
26-VIII, 1 P.M. Removed raisin.
27-VIII, 3 P.M. Tried on Control 2634 ; did not bite.
28-VIII, 2-30 P.M. Tried on Control 2634 ; did not bite.

29-VIII. Not tried.
30-VIII, 12-30 P.M. Tried on Control 2644 ; bit.
1-IX, 4-30 P.M. Tried repeatedly on Bull 2224 in chupper 52 ; did not bite.
3-IX, evening. Mosquito found dead.

*Mosquito 63.*

7-IX, 4 P.M. Found emerged. Enclosed with raisin.
9-IX, 2 P.M. Removed raisin
10-IX, 4 P.M. Tried on Control 2609 ; bit.
13-IX. Mosquito found dead.

*Mosquito 64.*

7-IX, 4 P.M. Found emerged. Enclosed with raisin.
9-IX, 2 P.M. Removed raisin.
10-IX, 4 P.M. Tried on Control 2609 ; did not bite.
12-IX, 11 A.M.—2 P.M. Tried on Control 2610 ; bit.
14-IX, 4-30 P.M. Tried on Bull 2224 in chupper 52 ; did not bite.
15-IX, 11-30 A.M. Ditto. Ditto.
16-IX, 10-30 A.M. Ditto. Ditto.
17-IX, 10-20—11-30 A.M. Tried on Bull 2224 in chupper 52 ; bit readily.
18-IX, 9-50 A.M. Tried repeatedly on Bull 2224 in chupper 52 ; bit.
19-IX, 10—11-10 A.M. Tried repeatedly and at intervals on Bull 2224 in chupper 52 ; did not bite. Abdomen of mosquito distended.
20-IX, 10 A.M. Tried on Bull 2224 in chupper 52 ; bit readily.
21-IX, 10-11 A.M. Tried on Bull 2224 in chupper 52 ; could not ascertain whether it had fed, as the day was cloudy and dark and contents of abdomen could not be seen with lens.
22-IX, 10—11-40 A.M. (Weather very chilly and cyclonic.) Tried on Bull 2224 in chupper 52 ; bit readily.
23-IX. Not tried. Weather very bad and continuous leakage in chupper 52.
24-IX, 10—noon Tried on Bull 2242 in chupper 53 ; did not bite.
25-IX, 10-30 A M.—1-30 P.M. Tried on Bull 2242 in chupper 53 ; did not bite.
26-IX, 10-15 A.M.—11-15 A.M. Tried on Bull 2242 in chupper 53 ; did not bite.
27-IX, 11-35 A.M.—12-10 P.M. Mosquito very thin. Tried on Bull 2242 in chupper 53 ; bit.
28-IX, 10-30—11-30 A.M. Tried at intervals on Bull 2242 in chupper 53 ; bit.
29-IX, 11-35 A.M.—noon. Tried on Bull 2242 in chupper 53 ; bit readily.
30-IX, 10-30—11-16 A.M. (Weather rainy and dark.) Tried on Bull 2242 in chupper 53 ; bit (?).
1-X, 10-30—noon. (Weather chilly and windy.) Tried at intervals on Bull 2242 in chupper 53 ; did not bite. Tried in the open as inside of chupper wet and muddy owing to previous day's rain.

2-X. Not recorded.
3-X, 11-40 A.M.—12-5 P.M. (Weather rainy and somewhat dark.) Tried at intervals on Bull 2631 (young bull) in chupper 54; bit.
4-X, 11-30—11-50 A.M. (Weather somewhat dark.) Tried at intervals on Bull 2631 (young bull) in chupper 54; did not bite.
5-X, 10-30 A.M. (Weather chilly; light changing.) Tried at intervals on Bull 2631 in chupper 54; did not bite.
6-X, 10-21—11-30 A.M. (Weather chilly; light changing.) Tried at intervals on Bull 2631 in chupper 54; did not bite.
7-X, 10-56—11-12 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; bit.
8-X, 10-37—11-5 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; bit.
9-X, 10-30—11 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; did not bite, but made attempts to puncture skin. Bull difficult to immobilize.
10-X, 10-35—11-15 A.M. (Weather chilly, but sunny.) Tried at intervals on Bull 2631 in chupper 54; did not bite.
11-X, 10-36 A.M. Mosquito found dead.

*Mosquito 65.*

7-IX, 4 P.M. Found emerged. Enclosed with raisin.
9-IX, 2 P.M. Removed raisin.
10-IX, 4 P.M. Tried on Control 2609; did not bite.
12-IX, 11 A.M.—2 P.M. Tried on Control 2610; bit.
14-IX, 4-30—5-30 P.M. Tried on Bull 2224 in chupper 52; bit readily.
15-IX, 10-15—11-30 A.M. Tried at intervals on Bull 2224 in chupper 52; did not bite.
16-IX, 10-30 A.M. Tried on Bull 2224 in chupper 52; bit readily.
17-X, 10-2A.M. Tried on Bull 2224 in chupper 52; bit.
18-IX, 9-50 A.M. Tried on Bull 2224 in chupper 52; bit.
19-IX, 10—11-10 A.M. Tried on Bull 2224 in chupper 52; bit readily.
20-IX, 10 A.M. Tried on Bull 2224 in chupper 52; bit readily. Excreted blood.
21-IX, 10-11 A.M. Tried on Bull 2224 in chupper 52; could not ascertain whether it had fed, as the day was very dark.
22-IX, 10—11-40 A.M. Tried on Bull 2224 in chupper 52; bit. Excreted blood.
23-IX, Not tried. Weather very bad and continuous leakage in chupper.
24-IX Not recorded.
25-IX, 10-30—1 P.M. Tried on Bull 2242 in chupper 53; did not bite.
26-IX, 10-15—11-15 A.M. Tried on Bull 2242 in chupper 53; bit readily.
27-IX, 11-35—12-10, Tried on Bull 2242 in chupper 53; did not bite.

28-IX, 10-30—11-30 A.M. Tried at intervals on Bull 2242 in chupper 53 ; bit readily when tried second time.

29-IX, 11-35 A.M.—noon. Tried on Bull 2242 in chupper 53 ; bit.

30-IX, 10-30—11-16 A.M. (Weather dark and rainy.) Tried on Bull 2242 in chupper 53 ; did not bite.

1-X, 11-30 A.M. Tried at intervals on Bull 2242 in chupper 53 ; did not bite. Tried in the open, as inside of chupper wet and muddy owing to previous day's rain.

2-X, Not recorded.

3-X, 11-40 A.M.—12-5 P.M. (Weather somewhat cloudy.) Tried at intervals on Bull 2631 in chupper 54 ; did not bite.

4-X, 11-30 A.M. Ditto. Ditto.

5-X, 10-30 A.M. (Weather chilly ; light changing.) Tried on Bull 2631 in chupper 54 ; bit readily.

6-X, 10-21—11-30 A.M. (Weather chilly ; light changing.) Tried on Bull 2631 in chupper 54 ; bit.

7-X, 10-56—11-12 A.M. (Light changing.) Tried on Bull 2631 in chupper 54 ; bit, but was not fully gorged.

8-X, 10-37—11-50 A.M. (Weather sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54 ; did not bite.

9-X, 10-30 A.M. (Weather sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54 ; did not bite.

10-X, 10-35—11-15 A.M. (Weather sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54 ; did not bite.

11-X, 10-36 A.M. Mosquito found dead.

*Mosquitos 66 to 71.*

8-IX, 5 P.M. Nos. 66 and 67 found emerged. Enclosed with raisins singly in tubes.

9-IX, 2 P.M. Raisins removed. Nos. 68, 69, 70 and 71 found emerged. Enclosed singly with raisins.

10-IX, 4 P.M. Raisins removed. Tried Nos. 66 and 67 only on Control 2609 ; did not bite.

12-IX, 11 A.M.—2 P.M. Tried on Control 2610 ; all the six mosquitos bit.

14-IX, 4-30—5-30 P.M. Tried on Bull 2224 in chupper 52. No. 70 bit but was not fully gorged ; No. 71 punctured but did not imbibe blood ; the rest did not bite.

15-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52. Nos. 66, 69, 70 and 71 did not bite ; No. 68 bit readily ; No. 67 bit when tried at intervals.

16-IX, 10-30 A.M. Tried on Bull 2224 in chupper 52. Nos. 67, 68, 70 and 71 did not bite ; No. 66 bit readily but was not fully gorged ; No. 69 bit readily.

17-IX, 10-30—11-30 A.M. Tried on Bull 2224 in chupper 52. No. 66 did not bite; Nos. 67, 69, 70 and 71 bit readily; No. 68 bit readily but was not fully gorged.

18-IX, 9-50 A.M. Tried on Bull 2224 in chupper 52. Nos. 66, 67, 69 did not bite; No. 71 bit readily; Nos. 68 and 70 bit, but not readily.

19-IX, 10—11-10 A.M. Tried on Bull 2224 in chupper 52. Nos. 68 and 69 did not bite; Nos. 67 and 71 bit readily; No. 70 bit when tried at intervals. No. 66 found dead.

20-IX, 10 A.M. Records obliterated by a downpour of rain whilst the feeding work was being carried on in a chupper.

21-IX, 10-11 A.M. Tried Nos. 67, 68, 69, 70 and 71 on Bull 2224 in chupper 52, but could not ascertain whether they had fed, as the day was dark.

22-IX, 10—11-40 A.M. (Weather chilly and cyclonic.) Tried on Bull 2224 in chupper 52. Nos. 67, 68 and 69 did not bite; Nos. 70 and 71 bit, but not readily.

23-IX. Not tried, as weather extremely bad and continuous leakage in chupper.

24-IX, 8 A.M. No. 69 found dead.

Noon. Tried on Bull 2242 in chupper 53; none bit.

25-IX, noon. Tried on Bull 2242 in chupper 53; none bit.

26-IX, 10-15—11-15 A.M. Ditto.

27-IX, 11-35 A.M.—12-10 P.M. Tried No. 67 on Bull 2242 in chupper 53; did not bite. Nos. 68, 70 and 71 dead.

*N.B.* The following observations relate to No. 67.

28-IX, 10-30—11-30 A.M. Tried on Bull 2242 in chupper 53; did not bite.

29-IX, noon. Tried on Bull 2242 in chupper 53; did not bite.

30-IX, 10-30—11-16 A.M. (Weather rainy and dark.) Tried on Bull 2242 in chupper 53; did not bite.

1-X, noon. Tried at intervals on Bull 2242 in chupper 53; did not bite. Tried in the open, as interior of chupper wet and muddy.

2-X. Not recorded.

3-X, 11-40 A.M.—12-5 P.M. (Rainy and somewhat cloudy.) Tried at intervals on Bull 2631 in chupper 54; bit.

4-X, 11-30 A.M. (Somewhat cloudy.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

5-X, 10-30 A.M. (Chilly; light changing.) Tried on Bull 2631 in chupper 54 bit readily.

6-X, 10-21—11-30 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

7-X, 11 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

8-X, 10-37—11-5 A.M. (Sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

9-X, 10-30—11 A.M. (Chilly and sunny.) Tried on Bull 2631 in chupper 54; did not bite. Bull difficult to immobilize.

10-X, 10-35—11-15 A.M. (Chilly and sunny.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

11-X, 10-36—11-30 A.M. (Chilly and sunny.) Tried at intervals on Bull 2631 in chupper 54; bit.

12-X, 10-45—11-22 A.M. (Chilly and sunny.) Tried on Bull 2631 in chupper 54; bit, but not readily.

13-X, 10-15—10-45 A.M. (Chilly and sunny.) Tried at intervals on Bull 2631 in chupper 54; bit.

14-X, 10—10-42 A.M. (Chilly and sunny.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

15-X, 10-30 A.M. (Light changing.) Tried on Bull 2631 in chupper 54; did not bite. Fair trial could not be given owing to *gwalla* (cattle-attendant) being bitten by the bull.

16-X, 9-50—10-30 A.M. (Light changing). Tried at intervals on Bull 2631 in chupper 54; bit, but did not take a full feed.

17-X, 9-40—10 A.M. (Sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

18-X, 10-10—10-50 A.M. (Sunny and chilly.) Tried at intervals on Bull 2631 in chupper 54; did not bite.

19-X, 9-50—10-20 A.M. (Very chilly; light changing.) Tried on Bull 2631 in chupper 54; did not bite. Mosquito fairly active.

20-X, 9-50—10-5 A.M. (Chilly and sunny.) Tried on Bull 2631 in chupper 54; did not bite. Mosquito sluggish.

21-X, 9-25 A.M. Mosquito dead.

*Mosquitos 72–76.*

9-IX, 2 P.M. No. 72 emerged. Enclosed with raisin.

10-X, 4 P.M. Removed raisin. Nos. 73, 74, 75 and 76 emerged. Enclosed singly in tubes with raisins.

12-IX, 2 P.M. Removed raisins.

13-IX, 11 A.M.—1 P.M. Tried on Control 2610; all bit.

14-IX, 4-30—5-30 P.M. Tried on Bull 2224 in chupper 52. Nos. 72, 73 and 75 did not bite; Nos. 74 and 76 bit readily.

15-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52. Nos. 75 and 76 bit when tried at intervals (abdomen of No. 76 already distended; the rest did not bite.

16-IX, 10-30 A.M. Tried on Bull 2224 in chupper 52; none bit.

17-IX, 10-20—11-20 A.M. Tried on Bull 2224 in chupper 52. Nos. 72, 73, 75 and 76 did not bite; No. 74 bit readily.

18-IX, 9-50 A.M. Tried on Bull 2224 in chupper 52. No. 72 moribund (dead short after); No. 74 bit readily; the rest did not bite.

19-IX, 10—11-10 A.M. Tried on Bull 2224 in chupper 52. Nos. 74, 75 and 76 bit readily (abdomen of No. 76 already distended); No. 73 did not bite.

20-IX, 10 A.M. Tried on Bull 2224 in chupper 52. Nos. 73 and 76 bit readily; the rest did not bite although tried at intervals.

21-IX, 10—11 A.M. (Dark and rainy.) Tried on Bull 2224 in chupper 52, but could not ascertain whether any had fed, as the day was dark.

22-IX, 10—11-40 A.M. (Dark, chilly and rainy.) Tried on Bull 2224 in chupper 52. Nos. 74 and 75 bit readily; the rest did not bite.

23 IX. Not tried, as weather extremely bad and continuous leakage in chupper.

24-IX, 10—11-15 A.M. Tried on Bull 2224 in chupper 52. No. 74 bit readily; No. 76 bit readily when tried a second time; the rest did not bite.

25-IX. Not recorded.

26-IX, 10-15—11-15 A.M. Tried on Bull 2242 in chupper 53. No. 74 bit readily; No. 76 bit readily when tried a second time; the rest did not bite, although tried at intervals.

27-IX, 11-35 A.M.—12-10 P.M. Tried on Bull 2242 in chupper 53. No. 76 bit, but not readily; the rest did not bite. No. 73 found dead.

28-IX, 10-30—11-30 A.M. Tried on Bull 2242 in chupper 53. No. 74 punctured, but did not imbibe blood, although tried at intervals; No. 75 did not bite, although tried at intervals. No. 76 moribund.

29-IX, 11-35 A.M.—noon. Tried on Bull 2242 in chupper 53. No. 74 bit readily; No. 75 did not bite.

30-IX, 10-30—11-16 A.M. (Rainy and dark.) Tried on Bull 2242 in chupper 53; none bit.

1-X, 11-30 A.M. (Windy and chilly.) Tried No. 74 at intervals on Bull 2242 in chupper 53; did not bite. Tried in the open using an umbrella to regulate light, as interior of chupper wet and muddy. No. 75 moribund.

2-X, 10-50—11-53 A.M. (Somewhat cloudy.) Tried No. 74 at intervals on Bull 2242 in chupper 53; did not bite. Bull difficult to immobilize. No. 75 dead.

3-X, 11-40 A.M.—12-5 P.M. (Light changing; rainy.) Tried No. 74 on Bull 2631 in chupper 54; did not bite. Mosquito extremely thin and moribund.

*Mosquito 77.*

11-IX, 3-30 P.M. Enclosed 12 newly-emerged females in a large bottle with raisins.

13-IX, 3-45 P.M. Removed raisins.

14-IX, Not tried.

15-IX. Ditto.

16-IX, noon. Tried on Control 2724; none bit. Enclosed the mosquitos singly in tubes and tried again; one only bit, this being numbered 77.

17-IX, 10-20—11-20 A.M. Tried on Bull 2224 in chupper 52 ; bit readily.
18-IX, 9-30 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
19-IX, 10—11-10 A.M. Tried on Bull 2224 in chupper 52 ; bit readily.
20-IX, 10 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
21-IX, 10—11 A.M. Tried on Bull 2224 in chupper 52, but could not ascertain whether it had fed, owing to insufficient light, as the day was cloudy
22-IX, 10—11-40 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
23-IX. Not tried owing to bad weather and leakage in chupper.
24-IX, 10—11-15 A.M. Tried on Bull 2224 in chupper 52 ; did not feed, but punctured skin.
25-IX, 10-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52 ; bit readily when tried a second time.
26-IX, 10-15—11-15 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
27-IX, 10-15—11-20 A.M. Tried on Bull 2224 in chupper 52 ; bit.
28-IX, 10-30—11-30 A.M. Tried on Bull 2242 in chupper 53 ; did not bite.
29-IX, 11-35 noon. Tried on Bull 2242 in chupper 53 ; bit readily.
30-IX, 10-30—11-16 A.M. (Cloudy and rainy.) Tried on Bull 2242 in chupper 53 ; bit readily.
1-X, 11-30 A.M.—noon. (Chilly.) Tried outside under umbrella (as chupper wet and muddy) on Bull 2242, chupper 53 ; did not bite.
2-X, 11 A.M. (Cloudy.) Tried at intervals on Bull 2242 in chupper 53 ; did not bite. Bull difficult to immobilize.
3-X, 10-48—11-30 A.M. Tried on Bull 2242 in chupper 53 ; bit.
4-X, 10-45—11-30 A.M. Tried on Bull 2242 in chupper 53 ; did not bite, although punctured skin. Abdomen of mosquito already distended.
5-X, 10-30—11-10 A.M. (Cloudy and rainy.) Tried at intervals on Bull 2242 in chupper 53 ; bit.
6-X, 10-30—11-15 A.M. (Light changing.) Tried on Bull 2631 in chupper 54 ; did not bite.
7-X, 10-56—11-12 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54 ; bit.
8-X, 10-37—11-5 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54 ; punctured, but did not feed. Abdomen of mosquito already distended.
9-X, 10-30—11 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; punctured but did not feed.
10-X, 10-35—11-15 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit readily but did not take a full feed.
11-X, 10-36—11-20 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit.
12-X, 10-45—11-22 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; punctured but did not feed.
13-X, 10-15—10-45 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit.

14-X, 10—10-42 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; punctured but did not feed.
15-X, 10-30 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54; bit, but did not take a full feed.
16-X, 9-50—10-30 A.M. (Light changing.) Tried on Bull 2631 in chupper 54; bit readily.
17-X, 9-40—10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; did not bite.
18-X, 10-30—10-50 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; bit. Abdomen already distended.
19-X, 9-50—10-20 A.M. (Light changing.) Tried on Bull 2631 in chupper 54; did not bite. Mosquito sluggish.
20-X, 9-50—10-5 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; did not bite.
21-X. Mosquito found dead.

*Mosquitos 77 (a) and 84.*

11-IX, 3-30 P.M. Enclosed 12 newly-emerged mosquitos in a large bottle with raisins.
13-IX. Removed raisins.
14-IX. Not tried.
15-IX. Ditto.
16-IX, noon. Tried on Control 2724; none bit. Enclosed the mosquitos singly in tubes and tried again on Control 2724; only one bit (No. 77).
17-IX, noon. Tried on Control 2728; one only bit (No. 77 (*a*)). As the remaining mosquitos looked very sluggish, put them all together in a small bottle with raisins.
18-IX, 11-30 A.M.—1-30 P.M. Tried on Control 2770; none bit, but mosquitos sprightly.
19-IX, noon—1-30 P.M. Tried repeatedly and at intervals on Control 2770; one only bit (No. 84).

*Mosquitos 78—83.*

12-IX, 5-30 P.M. Enclosed 8 newly-emerged females in a large bottle with raisins.
13-IX, 3-50 P.M. Added 3 more females.
15-IX, 4-30 P.M. Added 1 more female. Changed raisins as they were all covered over with fungus.
17-IX, 4-30 P.M. 1 dead, 1 moribund. Enclosed the rest singly in tubes without raisins.
18-IX, 11 A.M.—2 P.M. Tried on Control 2770; 6 bit. These were numbered 78—83.
19-IX. Not tried as mosquitos still distended with blood.

*Mosquitos 77(a), 78, 79, 80, 81, 82, 83 and 84.*

For early history see above.

20-IX, 10 A.M. Tried on Bull 2224 in chupper 52. Nos. 77 (*a*), 78, 79, 82, 83 and 84 bit readily; the rest did not bite.

21-IX, 10—11 A.M. Tried on Bull 2224 in chupper 52; No. 78 bit readily, but the rest could not be properly examined owing to insufficient light.

22-IX, 10—11-10 A.M. (Chilly, rainy and cloudy.) Tried on Bull 2224 in chupper 52. No. 77(*a*) bit readily; No. 80 bit, but not readily; No. 81 punctured skin, but did not draw blood; the rest did not bite. Abdomen of Nos. 82 and 83 already distended.

23-IX. Not tried, owing to bad weather and leakage in chupper.

24-IX, 10—11-15 A.M. Tried on Bull 2224 in chupper 52. Nos. 79, 80 and 81 bit readily, but No. 81 drew blood at repeated efforts; No. 82 punctured but did not draw blood; the rest did not bite. Abdomen of Nos. 82 and 83 already distended.

25-IX, 10-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52. Nos. 80 and 83 bit readily; No. 81 bit but not readily; Nos. 77(*a*) and 78 bit when tried at intervals; the rest did not bite.

26-IX, 11-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52. No. 83 bit readily. No. 82 bit, but not readily and took a long time to feed; Nos. 78 and 80 bit readily when tried a second time; No. 79 bit, but not readily, when tried at intervals; the rest did not bite.

27-IX, 10-15—11-21 A.M. No. 77 (*a*) dead. Tried the rest on Bull 2224 in chupper 52. Nos. 80 and 82 bit readily; No. 78 bit, but not readily; No. 81 punctured but did not draw blood (? fed slightly); the rest did not bite.

28-IX, 11-30 A.M.—12-23 P.M. Tried on Bull 2224 in chupper 52. No. 84 bit, but not readily; Nos. 80 and 82 punctured skin, but did not draw blood (?No. 82 fed slightly); the rest did not bite. No. 82 excreted blood. Abdomen of No. 80 already distended.

29-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52. Nos. 78, 83 and 84 bit, but not readily; No. 81 bit when tried at intervals (it took a long time to draw blood and brushed proboscis now and then); the rest did not bite. Abdomen of No. 82 already distended. No. 79 thin and moribund.

30-IX, 11-35—11-45 A.M. (Cloudy.) Tried only No. 84 on Bull 2224 in chupper 52; did not bite. Tried the rest on Bull 2242 in chupper 53; it could not be definitely ascertained whether they had bitten. Abdomen of Nos. 78 and 83 distended. No. 79 still alive, although moribund.

1-X, 11-30 A.M.—Noon. (Cloudy.) Tried (outside, under umbrella, as chupper wet and muddy) on Bull 2242, chupper 53; none bit. No. 79 dead.

2-X, 10-50—11-53 A.M. (Cloudy.) Tried on Bull 2242 in chupper 53. No. 80 bit readily ; Nos. 81 and 84 bit, but not readily. Bull difficult to immobilize.

3-X, 10-48—11-30 A.M. (Cloudy.) Tried on Bull 2242 in chupper 53. No. 83 bit readily ; No. 81 bit, but not readily ; Nos. 80 and 82 bit when tried at intervals ; the rest did not bite although tried at intervals.

4-X, 10-45—11-30 A.M. (Sunny.) Tried on Bull 2242 in chupper 53. Nos. 81 and 83 bit readily ; No. 78 bit when tried at intervals ; No. 82 bit when tried at intervals, but did not take a full feed ; No. 80 punctured but did not draw blood ; No. 80 would probably have fed but bull became refractory, just at the moment the mosquito was about to bite ; No. 84 thin and did not bite.

5-X, 10-30—11-10 A.M. (Cloudy.) Tried on Bull 2242 in chupper 53. No. 78 bit, but not readily ; No. 83 bit when tried at intervals ; the rest did not bite, although tried at intervals.

6-X, 10-30—11-15 A.M. (Light changing.) Tried at intervals on Bull 2242 in chupper 53 ; none bit. Abdomen of No. 78 already distended. Nos. 80 and 82 moribund.

7-X, 10-30—10-48 A.M. (Sunny.) Nos. 80, 82 and 83 dead. Tried the rest, at intervals, on Bull 2242 in chupper 53. No. 81 bit readily ; No. 84 bit, but not readily ; No. 78 did not bite, although tried at intervals.

8-X, 10-37—11-5 A.M. (Sunny.) Tried only No. 84, at intervals, on Bull 2242 in chupper 53 ; did not bite. Tried the rest on Bull 2631 in chupper 54. No. 81 bit readily ; No. 78 bit when tried at intervals.

9-X, 10-30—11 A.M. (Sunny.) Tried on Bull 2631 in chupper 54. Nos. 78 and 81 bit readily (abdomen of No. 81 already distended) ; No. 84 bit, but not readily.

10-X, 10-35—11-15 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; No. 84 bit but none of the rest bit, although tried at intervals.

11-X, 10-36—11-20 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; No. 78 bit readily, but none of the rest bit although tried at intervals.

12-X, 10-45—11-22 A.M. (Sunny.) Tried on Bull 2231 in chupper 54. Nos. 81 and 84 bit readily, but No. 84 did not take a full feed ; No. 78 did not bite, although tried at intervals. Abdomen of No. 78 already distended.

13 X, 10-45—11-45 A.M. (Sunny.) Tried on Bull 2631 in chupper 54. Nos. 78 and 84 bit, but not readily ; No. 81 bit readily when tried a second time.

14-X, 10—10-42 A M. (Sunny.) Tried on Bull 2631 in chupper 54. No. 81 bit, but not readily ; No. 78 bit readily when tried a second time ; No. 84 did not bite, although tried at intervals.

15-X, 10-30 A.M. (Sunny.) Tried at intervals on Bull 2631 in chupper 54 ; none bit. A fair trial could not be given.

16-X, 9-50—10-30 A.M. (Light changing.) Tried on Bull 2631 in chupper 54; Nos. 78 and 81 bit when tried at intervals, but No. 81 did not take a full feed. No. 84 moribund.

17-X, 9-40—10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54. No. 81 bit readily; No. 78 did not bite, although tried at intervals. No. 84 dead.

18-X, 10-10—10-50 A.M. (Sunny.) Tried at intervals on Bull 2631 in chupper 54. No. 81 punctured but did not draw blood; No. 78 did not bite, although tried at intervals.

19-X, 9-50—10-30 A.M. (Light changing.) Tried on Bull 2631 in chupper 54. No. 78 bit readily, but did not take a full feed (bull refractory all the time and the mosquito took a long time to draw blood); No. 81 very sluggish, but escaped through torn muslin.

20-X, 9-50—10-5 A.M. (Sunny.) Tried No. 78 on Bull 2631 in chupper 54; did not bite.

21-X. No. 78 dead.

*Mosquitos 85, 86 and 87.*

17-IX, 4 P.M. Enclosed 7 newly-emerged mosquitos singly in tubes with raisins.

18-IX, 5 P.M. Removed raisins.

19-IX, 11 A.M.—1 P.M. 3 only alive. Tried these on Control 2770; all bit.

21-IX, 10-11 A.M. Tried on Bull 2224 in chupper 52. No. 87, bit readily; the rest could not be properly examined owing to insufficient light as the day was cloudy.

22-IX, 10—11-45 A.M. (Cloudy and rainy.) Tried on Bull 2224 in chupper 52. No. 87 bit readily; the rest did not bite although tried at intervals.

23-IX. Not tried owing to bad weather and leakage in chupper.

24-IX, 10—11-15 A.M. Tried on Bull 2224 in chupper 52. No. 87 bit readily when tried a second time (although its abdomen was already distended); No. 86 tried at intervals and punctured, but did not draw blood; No. 85 did not bite, although tried at intervals.

25-IX, 10-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52. No. 87, bit readily; the rest did not bite, although tried at intervals.

26-IX, 11-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52. No. 87 bit readily; No. 86 bit readily when tried a second time; No. 85 did not bite, although tried at intervals.

27-IX, 10-15—11-21 A.M. Tried on Bull 2224 in chupper 52. No. 87 bit readily (although its abdomen was already distended); No. 86 bit, but not readily; No. 85 bit, but not readily.

28-IX, 11-30 A.M.—12-23 P.M. Tried at intervals on Bull 2224 in chupper 52; none bit. No. 85 moribund.

29-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52. No. 87 bit, but not readily (its abdomen already distended); No. 86 did not bite, although tried at intervals. No. 85 still moribund.

30-IX, 10-35—11-25 A.M. (Cloudy.) Nos. 85 and 86 dead. Tried No. 87 on Bull 2224 in chupper 52 ; did not feed (? fed slightly).

*N.B.* The following observations relate to No. 87 only.

1-X, 10-30 A.M. (Cloudy.) Tried on Bull 2224 in chupper 52 ; did not bite (? fed slightly).

2-X, 10-50—11-53 A.M. (Cloudy.) Tried on Bull 2242 in chupper 53 ; punctured, but did not draw blood.

3-X, 10-48—11-30 A.M. (Cloudy.) Tried at intervals on Bull 2242 in chupper 52 ; bit, although abdomen already distended.

4-X, 10-45—11-30 A.M. (Sunny.) Tried at intervals on Bull 2242 in chupper 53 ; did not draw blood, although punctured skin. Abdomen already distended. Tried again on Bull 2242 after some time ; bit.

5-X, 10-30—11-10 A.M. (Cloudy.) Tried at intervals on Bull 2242 in chupper 53 ; did not bite ( ? fed slightly.) Abdomen already distended.

6-X, 10-30—11-15 A.M. (Light changing.) Tried on Bull 2242 in chupper 53 ; punctured, but did not draw blood. Abdomen already distended.

7-X, 10-30—10-48 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; punctured, but did not draw blood. Took a full feed when tried again after a whil .

8-X, 10-26—10-35 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit readily.

9-X, 10-30—11 A.M. (Sunny.) Tried at intervals on Bull 2631 in chupper 54 ; did not draw blood, although punctured skin. Abdomen already distended.

10-X, 10-35—11 A.M. (Sunny.) Ditto.

11-X, 10-36—11-20 A.M. (Sunny.) Tried at intervals on Bull 2631 in chupper 54 ; bit.

12-X, 10-45—11-22 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit readily.

13-X, 10-15—10-45 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit readily, although took a long time to draw blood.

14-X, 10—10-42 A.M. (Sunny). Tried at intervals on Bull 2631 in chupper 54 ; punctured every time applied on animal, but did not draw blood.

15-X, 10-30 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54 ; did not bite. A fair trial could not be given.

16-X, 9-50—10-30 A.M. (Light changing.) Tried at intervals on Bull 2631 in chupper 54 ; punctured, but did not draw blood. Abdomen already distended.

17-X, 9-40—10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54 ; bit readily, although abdomen already distended.

18-X, 10—10-50 A.M. (Sunny.) Tried at intervals on Bull 2631 in chupper 54 ; bit although abdomen already distended.

19-X, 9-50—10-20 A.M. (Light changing.) Tried on Bull 2631 in chupper 54 ; did not draw blood, although punctured skin. Abdomen already distended.

20-X, 9-50—10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; bit readily but did not take a full feed. Abdomen already distended.

21-X, 9-25 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; bit readily, although abdomen already distended.

22-X, 10 A.M. (Sunny.) Ditto.

23-X, 10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; did not bite. Abdomen already distended.

24-X, 10 A.M. (Sunny.) Tried on Bull 2631 in chupper 54; bit readily, although abdomen already distended.

25-X, 10 A.M. (Sunny). Tried on Bull 2631 in chupper 54; punctured, but did not draw blood. Abdomen already distended.

Noon. Mosquito moribund.

1 P.M. Mosquito dead.

*Mosquito 88.*

19-IX, 4-30 P.M. Emerged. Enclosed with raisin.

21-IX, 2-15 P.M. Removed raisin.

22-IX, 1 P.M. Tried on Control 2982; punctured, but did not draw blood.

24-IX, 3-45 P.M. Tried on Control 2655; bit.

25-IX, 10 A.M.—1 P.M. Tried on Bull 2224 in chupper 52; bit readily when tried a second time.

26-IX, 11-30 A.M.—1 P.M. Tried on Bull 2224 in chupper 52; bit readily, although abdomen already distended.

27-IX, 10-15—11-21 A.M. Tried on Bull 2224 in chupper 52; bit readily.

28-IX, 11-30 A.M.—12-23 P.M. Tried at intervals on Bull 2224 in chupper 52; punctured but did not draw blood. Abdomen already distended.

29-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52; bit readily.

30-IX, 11-35—11-45 A.M. (Cloudy.) Tried on Bull 2224 in chupper 52; bit, but not readily.

1-X, 10-30—11-30 A.M. (Cloudy and rainy.) Tried at intervals on Bull 2224 in chupper 52; did not bite. Abdomen already distended.

2-X, 10-50—11-53 A.M. (Cloudy.) Tried at intervals on Bull 2224 in chupper 52; did not bite. Bull difficult to immobilize.

3-X, 10-15—11-25 A.M. (Light changing.) Tried on Bull 2224 in chupper 52; bit, but not readily.

4-X, 10-30—10-40 A.M. (Sunny.) Tried on Bull 2224 in chupper 52; bit readily when tried a second time.

5-X, 10—10-24 A.M. (Cloudy.) Tried at intervals on Bull 2224 in chupper 52; bit, although abdomen already distended. It first punctured for some-time without drawing blood.

6-X, 10-30—11-15 A.M. (Light changing.) Tried repeatedly on Bull 2242 in chupper 53; did not bite.

7-X, 10-30—10-48 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit, but not readily. Abdomen already distended.
8-X, 10-26—10-35 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit, but not readily. Abdomen already distended.
9-X, 10-25 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit readily.
10-X, 10-30 A.M. (Sunny.) Tried at intervals on Bull 2242 in chupper 53 ; did not bite. Abdomen already distended.
11-X, 10-30 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit readily, although abdomen already distended.
12-X. Mosquito dead.

*Mosquito 89.*

24-IX, 4-30 P.M. Emerged. Enclosed with raisin.
25-IX, 4-45 P.M. Removed raisin.
26-IX, 2 P.M. Tried on Control 2748 ; bit readily.
27-IX, 10-15—11-21 A.M. Tried on Bull 2224 in chupper 52 ; bit readily.
28-IX, 11-30 A.M.—12-23 P.M. Tried on Bull 2224 in chupper 52 ; punctured, but did not draw blood.
29-IX, 10-15—11-30 A.M. Tried on Bull 2224 in chupper 52 ; bit, but not readily.
30-IX, 10-35—11-15 A.M. Tried on Bull 2224 in chupper 52 ; did not bite.
1-X, 10-30—11-10 A.M. (Cloudy and rainy.) Tried on Bull 2224 in chupper 52 ; bit, but not readily.
2-X, 10-30—10-45 A.M. (Cloudy.) Ditto.
3-X, 10-15—10-25 A.M. (Light changing.) Tried at intervals on Bull 2224 in chupper 52 ; did not bite.
4-X, 10-20—10-40 A.M. (Sunny.) Tried on Bull 2224 in chupper 52 ; bit, but not readily.
5-X, 10—10-24 A.M. (Cloudy.) Tried at intervals on Bull 2224 in chupper 52 ; did not bite.
6-X, 10—10-20 A.M. (Light changing.) Tried on Bull 2224 in chupper 52 ; bit when tried a second time, but did not take a full feed.
7-X, 10—10-14 A.M. (Sunny.) Tried on Bull 2224 in chupper 52 ; bit readily.
8-X, 10-26—10-35 A.M. (Sunny.) Tried at intervals on Bull 2242 in chupper 53 ; bit.
9-X, 10-25 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit readily.
10-X, 10-30 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; bit readily.
11-X, 10-30 A.M. (Sunny.) Tried on Bull 2242 in chupper 53 ; punctured, but did not draw blood. Bull refractory.
12-X. Mosquito dead.

*Mosquitos 90 and 91.*

26-IX, 4-30 P.M. Emerged. Enclosed with raisins singly in tubes.
27-IX, 2 P.M. Raisins not yet removed. Mosquitos kept in a cloth cage ever a basin of water.

28-IX, 1-30 P.M. Mosquitos fairly active.
29-IX, 1-8 P.M. Mosquitos sluggish.
29-IX, 1-48 P.M. Kept in incubator (28°C.).
4-30 P.M. Removed raisins. Kept in incubator (28°C.).
30-IX, 1-50 P.M. Mosquitos happy.
2-30 P.M. (Temperature in room, 13°C.) Tried on Control 2750; one bit readily. This was numbered 90.
2-30 P.M. Tried another at intervals (under identical conditions) on Control 2750; bit. This was numbered 91.

*Mosquito 92.*

27-IX, 4-20 P.M. Emerged. Enclosed in tube with raisin and kept in incubator.
28-IX, 3 P.M. Mosquito happy.
29-IX, 1 P.M. Ditto.
4-15 P.M. Removed raisin and placed back tube in incubator.
30-IX, 1-50 P.M. Mosquito happy.
3-30 P.M. Tried at intervals on Control 2750; bit.

*Mosquitos 90, 91, and 92*

For early history see above.
1-X, 10-30—11-10 A.M. (Cloudy.) Tried on Bull 2224 in chupper 52. Nos. 90 and 92 bit readily when tried a second time; No. 91 bit, but not readily, when tried a second time and did not take a full feed.
2-X, 10-20—10-45 A.M. (Cloudy.) Tried on Bull 2224 in chupper 52. Nos. 91 and 92 bit, but not readily and No. 91 did not take a full feed; No. 90 bit readily when tried a second time.
3-X, 10-20—10-40 A.M. (Sunny.) No. 91 dead. Tried Nos. 90 and 92 at intervals on Bull 2224 in chupper 52; none bit.
4-X, 10-20—10-40 A.M. (Sunny.) Ditto.
5-X, 10—10-24 A.M. (Cloudy.) Ditto.
6-X, 10—10-20 A.M. (Light changing.) Ditto.
7-X, 10—10-14 A.M. (Sunny.) Tried on Bull 2224 in chupper 52; No. 92 bit, but drew blood at repeated efforts (the muslin had to be stretched); No. 90 did not draw blood, but punctured every time it was applied on animal.
8-X, 10-20 A.M. (Sunny.) Tried at intervals on Bull 2224 in chupper 52; none bit.
9-X, 10-10 A.M. Ditto. Ditto.
10-X, 10-10 A.M. (Sunny.) No. 90 dead. Tried No. 92 on Bull 2224 in chupper 52; did not bite.
11-X, 10-15 A.M. (Sunny.) Tried No. 92 on Bull 2224 in chupper 52; bit readily.
12-X, 10-30 A.M. (Sunny.) Tried No. 92 at intervals on Bull 2242 in chupper 53; did not bite.

13-X, Not tried.
14-X, 10 A.M. (Sunny.) Tried No. 92 on Bull 2242 in chupper 53; did not bite. Mosquito moribund.
15-X. No. 92 dead.

*Mosquito 93.*

10-X, 1-15 P.M. Emerged. Enclosed with raisin, along with 2 more, in a large bottle and kept in incubator (28°C.).
11-X, noon. Mosquitos happy.
2 P.M. Tried on Control 2223; 1 bit readily. This was numbered 93 and was enclosed separately in tube.
2-5 P.M. The mosquito was carried about from place to place (being kept in pocket close to the person) during field work.
5 P.M. Mosquito found dead.

*Mosquito 94.*

13-X, 3 P.M. Enclosed 2 newly-emerged mosquitos in bottle with raisins and kept in incubator (28°C.).
14-X, 11-30 A.M. Both happy.
12-30 P.M. Tried on Control 2246 for a long time; did not bite.
15-X, 1-30 P.M. Tried on Control 2246; 1 only bit. This was enclosed separately in tube and numbered 94.
16-X and 17-X. Not tried.
18-X, 10—10-15 A.M. Tried at intervals on Bull 2224 in chupper 52; did not bite.
19-X. Mosquito dead.

*Mosquito 95. (One of the two mosquitos enclosed along with No. 93 on 10-X.)*

11-X. Tried on Control 2223; did not bite. Enclosed mosquito in a large bottle with raisins, and kept in incubator (28°C.).
12-X, 1-37 P.M. Mosquito happy.
13-X, 3 P.M. Ditto.
14-X, 11-30 A.M. Changed raisins as they were covered over with fungus.
12-30 P.M. Tried for a long time on Control 2246; did not bite.
15-X, 1-30 P.M. Tried repeatedly and at intervals on Control 2246; did not bite. Enclosed mosquito in tube, without raisin, and kept tube inverted over moist filter paper in incubator.
16-X, 3-22 P.M. Mosquito happy.
17-X, 10-49 A.M. Ditto.
18-X, 12-30 P.M. Tried at intervals on Control 2440; bit.
19-X, 9-30—9-45 A.M. (Sunny.) Tried on Bull 2224 in chupper 52; bit readily, although abdomen distended.
20-X. Mosquito found dead.

*Mosquito 96.* (*The mosquito enclosed along with No. 94 on 13-X.*)

15-X, 1-30 P.M. Tried repeatedly and at intervals on Control 2246; did not bite. Enclosed mosquito in tube without raisin, and kept tube inverted over moist filter paper in incubator.

16-X, 3-30 P.M. Mosquito happy.

17-X, 10-48 A.M. Ditto.

12-30 P.M. Tried at intervals on Control 2440; bit.

*Mosquito 97.*

15-X, 1-30 P.M. Enclosed 4 females and 1 male in a large bottle, with raisins, in incubator.

16-X, 3-28 P.M. All happy. Changed raisins as they were covered over with fungus.

17-X, 11 A.M. Male dead; the rest happy. No fungus on raisins yet.[1]

12-30 P.M. Enclosed the mosquitos singly in tubes and tried on Control 2440; 1 bit readily. This was numbered 97.

*Mosquitos 96 and 97.*

For early history see above.

18-X, 10—10-15 A.M. (Sunny.) Tried on Bull 2224 in chupper 52. No. 96 bit, but not readily; No. 97 did not bite, although tried at intervals.

19-X, 9-30—9-45 A.M. (Sunny.) Both the mosquitos very sluggish (apparently due to very low atmospheric temperature) and still distended with blood imbibed during their initial feed. Tried on Bull 2224 in chupper 52. No. 96 bit readily; No. 97 did not bite. Mosquitos regained activity shortly after being placed in contact with animal.

20-X. Both mosquitos dead.

*Mosquitos 98, 99 and 100.*

(*These were the three mosquitos enclosed with No. 97 on 15-X.*)

17-X, 11 A.M. All happy, but male dead.

12-30 P.M. Enclosed the mosquitos, singly, in tubes and tried on Control 2440; did not bite. Kept mosquitos in incubator.

18-X, 1 P.M. Not tried.

19-X, noon. Tried on Control 2627; all bit. Kept them in incubator without food, the tubes being kept inverted over moist filter paper.

22-X, 12-30 P.M. All happy.

23-X, 10-30 A.M. One dead; the rest happy.

14-X 10 A.M. The other two dead.

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[1]The relevancy of these remarks arises from the fact that the raisins (offered to the mosquitos to feed upon) were very quickly covered over with fungus when placed in the incubator, as compared with what occurred under the ordinary atmospheric conditions at Muktesar.

*Mosquitos 101 and 102.*

17-X, 1-12 P.M. Emerged. Enclosed, singly, in tubes, with raisins. Kept tubes inverted over moist filter paper in incubator.

18-X, 1 P.M. Both happy.

19-X, noon. Both happy. Tried on Control 2627; did not bite.

1-29 P.M. Removed raisins.

21-X, 10-20 A.M. Both happy.

11 A.M. Tried on Control 3181; both bit. These were numbered 101 and 102. Placed No. 101 in incubator over moist filter paper. No. 102 was kept in cupboard over moist muslin in room temperature.

22-X, 12-22 P.M. Mosquito in incubator quite active; that in cupboard very sluggish.

23-X, 10-30 A.M. Ditto. Ditto.

24-X, 10-12 A.M. Ditto. Ditto.

Enclosed both together in a large bottle with moist filter paper and raisins and kept bottle in incubator.

25-X, 11 A.M. Both happy.

26-X, 4 P.M. Both happy.

27-X, 2 P.M. Both happy. Changed raisins as they were mouldy.

28-X, 11-30 A M. Both happy.

29-X, noon. Both happy. Enclosed the two singly in tubes.

3-30 P.M. Tried on Bull 2224 in chupper 52; only 1 bit, but not readily and did not take a full feed. The mosquitos became extremely sluggish when taken out of the incubator (the day was cloudy and very chilly).

Experiments discontinued.

As will be seen from the foregoing, the feeding experiments may be divided into two series :—

Series I (1-VI to 13-VIII). There were 18 experimental bulls divided into two lots of 15 and 3. The mosquitos were tried on bulls of the first lot every 2 days since the date of infective feed, and every 4 days on bulls of the second lot, a mosquito being tried only one day on the same bull.

Series II (14-VIII to 29-X). There were only 3 experimental bulls—Nos. 2224, 2242, and 2631. Each mosquito was tried on Bull 2224 up to the 11th day from the date on which it sucked infected blood. From the 12th to the 19th day it was tried on Bull 2242, and from the 20th day onward, on Bull 2631. But here again, the feeding experiments may be divided into two categories :—

1 (14-VIII to 14-IX). The mosquitos were tried on bulls every 2 days.

2 (15-IX to 29-X). The mosquitos were tried on bulls every day.

Where a deviation from the above rules has occurred, or where a mosquito died during the progress of an experiment, the fact has been indicated in the protocols.

The feeding experiments may therefore be tabulated as follows (the tables show the frequency with which each mosquito bit)[1] :—

[1]All bulls were subsequently found on testing to have been actually susceptible to rinderpest.

## Table of feeding experiments on bulls.

*Series*

| Number of experimental animal. | Mosquito 1 | Mosquito 2 | Mosquito 5 | Mosquito 6 | Mosquito 8 | Mosquito 10 | Mosquito 11 | Mosquitos 12 * | Mosquitos 13* | Mosquito 14 | Mosquitos 14 (a)** | Mosquito 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2322 | — | — | — | — | — | — | — | — | + + | — | — | — |
| 2224 | — | + | — | + | — | Dead | — | — | + | + | — | — |
| 2223 | — | — | + | + | — | .. | + | + | + | — | + | — |
| 2245 | — | + | — | + | — | .. | — | — | one dead + | + | + | — |
| 2241 | + | — | — | + | — | .. | — | — | — | — | + | — |
| 2243 | — | + | — | + | — | .. | + | — | + | + | Not tried | — |
| 2240 | — | Moribund | — | + | — | .. | — | — | — | Dead | Not tried | Dead |
| 2246 | — | Dead | — | + | Dead | .. | — | — | Not tried | .. | — | .. |
| 2242 | — | .. | + | + | .. | .. | Dead | Dead | + | .. | — | .. |
| 2244 | — | .. | + | + | .. | .. | .. | .. | — | .. | + + | .. |
| 2348 | .. | .. | — | — | .. | .. | .. | .. | Dead | .. | + | .. |
| 2627 | .. | .. | | .. | .. | .. | .. | .. | .. | .. | + .. | .. |
| 2346 | .. | .. | Not tried | | .. | .. | .. | .. | .. | .. | + | .. |
| 2626 | .. | .. | | | .. | .. | .. | .. | .. | .. | .. | .. |
| 2349 | .. | .. | — | — | .. | .. | .. | .. | .. | .. | — | .. |
| 2599 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2351 | .. | .. | + | — | .. | .. | .. | .. | .. | .. | + | .. |
| 2347 | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | + | .. |
| 2614 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2623 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2440 | .. | .. | + | Dead | .. | .. | .. | .. | .. | .. | + | .. |
| 2442 | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | Dead | .. |
| 2631 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2439 | .. | .. | Dead | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2678 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

* Two mosquitos enclosed
** See schedule of experi

*I.*

| Mosquitos 15 (a)** | Mosquito 16 | Mosquito 17 * | Mosquito 18 | Mosquito 19 | Mosquito 20 | Mosquito 21 | Mosquito 22 | Mosquito 23 | Mosquito 24 | Mosquitos 25 * | Mosquito 27 | Mosquito 28 | Mosquito 29 | Mosquito 30 | Mosquito 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Not tried | — | — | — | + | — | + | + | + | — | — | + | + | + | + | + |
| .. | + | + | — | Not tried | Dead | + | + | + | + | + | + | + | Dead | + | + |
| .. | Not tried | Not tried | Not tried | + | .. | + | + | + | + | + + | — | + | .. | + | + |
| — | + | + one dead | + | — | .. | + | + | Dead | + | Dead | Dead | + | .. | + | Dead |
| — | — | — | Dead | Dead | .. | + | — | .. | — | .. | .. | + | .. | Dead | .. |
| Not tried | — | + | .. | .. | .. | + | + | .. | + | .. | .. | Dead | .. | .. | .. |
| — | + | — | .. | .. | .. | — | Dead | .. | + | .. | .. | .. | .. | .. | .. |
| — | — | — | .. | .. | .. | — | .. | .. | — | .. | .. | .. | .. | .. | .. |
| — | ? + | Dead | .. | .. | .. | Dead | .. | .. | Dead | .. | .. | .. | .. | .. | .. |
| Dead | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | Dead | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | ..

*Series*

| Number of experimental animal | Mosquito 32 | Mosquito 33 | Mosquito 34 | Mosquito 35 | Mosquito 36 | Mosquito 37 | Mosquito 38 | Mosquito 39 |
|---|---|---|---|---|---|---|---|---|
| 2222 | + | — | + | + | + | + | + | + |
| 2224 | + | + | + | + | + | + | Damaged | + |
| 2223 | — | Dead | + | + | + | + | .. | + |
| 2245 | — | .. | Dead | + | — | — | .. | — |
| 2241 | Dead | .. | .. | + | — | — | .. | — |
| 2243 | .. | .. | .. | — | Dead | — | .. | — |
| [illegible] | .. | .. | .. | — | .. | — | .. | — |
| 2246 | .. | .. | .. | + | .. | — | .. | Dead |
| 2242 | .. | .. | .. | + | .. | — | .. | .. |
| 2244 | .. | .. | .. | — | .. | — | .. | .. |
| 2348 / 2627 | .. | .. | .. | — | .. | .. | .. | .. |
| 2346 | .. | .. | .. | .. | .. | .. | .. | .. |
| 2626 | .. | .. | .. | + | .. | — | .. | .. |
| 2349 | .. | .. | .. | .. | .. | .. | .. | .. |
| 2590 | .. | .. | .. | — | .. | — | .. | .. |
| 2351 | .. | .. | .. | —* | .. | Moribund | .. | .. |
| 2347 | .. | .. | .. | Inoculated into Bull 2723 | .. | .. | .. | .. |
| 2614 | .. | .. | .. | | .. | .. | .. | .. |
| 2623 | .. | .. | .. | | .. | .. | .. | .. |
| 2440 | .. | .. | .. | | .. | .. | .. | .. |
| 2442 | .. | .. | .. | | .. | .. | .. | .. |
| 2631 | .. | .. | .. | | .. | .. | .. | .. |
| 2439 | .. | .. | .. | .. | .. | .. | .. | .. |
| 2678 | .. | .. | .. | .. | .. | .. | .. | .. |

* This observation is shown against Bull 2462

*I*—continued.

| Mosquito 40 | Mosquito 41 | Mosquito 42 | Mosquito 43 | Mosquito 44 | Mosquito 46 | Mosquito 47 | Mosquito 49 | Mosquito 50 | Mosquito 53 | Mosquito 54 | Mosquito 55 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | — | + | + | + | ? + | + | + | + | — | — |
| + | + | — | + | + | — | + | .. | | + | — | — |
| + | — | — | + | + | Not tried | + | + | | — | + | — |
| — | — | — | — | + | — | — | Escaped | | | | |
| + | — | + | + | + | — | — | .. | | | | |
| — | — | — | Dead | + | — | + | .. | | | | |
| — | — | — | .. | + | — | + | .. | | | | |
| + | Dead | + | .. | + | Dead | | .. | | | | |
| + | .. | + | .. | | .. | | .. | | | | |
| — | .. | + | .. | | .. | | .. | | | | |
| .. | .. | | .. | | .. | | .. | | | | |
| — | .. | | .. | | .. | | .. | | | | |
| Dead | .. | | .. | | .. | | .. | | | | |
| .. | .. | | .. | | .. | | .. | | | | |
| .. | .. | | .. | | .. | | .. | | | | |
| ..

*Series*

| No. of animal | Days | Mosquito 57 | Mosquito 58 | Mosquito 59 | Mosquito 60 | Mosquito 61 | Mosquito 62 |
|---|---|---|---|---|---|---|---|
| No. 2224 | 2nd day | — | + | + | — | + | — |
| | 4th day | — | — | — | + | + | Dead |
| | 6th day | — | — | + | — | — | .. |
| | 8th day | + | Dead | + | Dead | + | .. |
| | 10th day | — | .. | + | .. | Dead | .. |
| No. 2242 | 12th day | + | .. | Dead | .. | .. | .. |
| | 14th day | — | .. | .. | .. | .. | .. |
| | 16th day | — | .. | .. | .. | .. | .. |
| | 18th day | Dead | .. | .. | .. | .. | .. |
| No. 2631 | 20th day | .. | .. | .. | .. | .. | .. |
| | 22nd day | .. | .. | .. | .. | .. | .. |
| | 24th day | .. | .. | .. | .. | .. | .. |
| | 26th day | .. | .. | .. | .. | .. | .. |
| | 28th day | .. | .. | .. | .. | .. | .. |
| | 30th day | .. | .. | .. | .. | .. | .. |

*Series*

| No. of animal | Days | Mosquito 64 | Mosquito 65 | Mosquito 66 | Mosquito 67 | Mosquito 68 | Mosquito 69 | Mosquito 70 | Mosquito 71 | Mosquito 72 | Mosquito 73 | Mosquito 74 | Mosquito 75 | Mosquito 76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. 2224 | 1st day | Continued from last series | | | | | | | | — | — | + | — | + |
| | 2nd day | .. | .. | .. | .. | .. | .. | .. | .. | — | — | — | + | + |
| | 3rd day | — | — | — | + | + | — | — | — | — | — | — | — | — |
| | 4th day | — | + | + | — | — | + | — | — | — | — | + | — | — |
| | 5th day | + | — | — | + | + | + | + | + | D. | — | + | — | — |
| | 6th day | + | + | — | — | + | — | + | + | .. | — | + | + | + |
| | 7th day | — | + | D. | + | — | — | + | + | .. | + | — | — | + |
| | 8th day | + | + | Records illegible (See under date 20-IX) | | | | | | .. | ? | ? | ? | ? |
| | 9th day | ?+ | ?+ | .. | ? | ? | ? | ? | | .. | — | + | + | — |
| | 10th day | + | + | .. | — | — | N.T. | + | + | .. | N.T. | N.T. | N.T. | N.T. |
| | 11th day | N.T. | N.T. | .. | N.T. | N.T. | N.T. | N.T. | N.T. | .. | — | + | — | + |

*II.* (*1*)

| Mosquito 64 | Mosquito 65 | Mosquito 66 | Mosquito 67 | Mosquito 68 | Mosquito 69 | Mosquito 70 | Mosquito 71 |
|---|---|---|---|---|---|---|---|
| — | + | — | — | — | — | + | — |
| Continued to next series. | Continued to next series. | Continued to next series. | Continued to next series. | Continued to next series. | Continued to next series. | Continued to next series. | Continued to next series. |

*II.* (*2*)

| Mosquito 77 | Mosquito 77(a) | Mosquito 78 | Mosquito 79 | Mosquito 80 | Mosquito 81 | Mosquito 82 | Mosquito 83 | Mosquito 84 | Mosquito 85 | Mosquito 86 | Mosquito 87 | Mosquito 88 | Mosquito 89 | Mosquito 90 | Mosquito 91 | Mosquito 92 | Mosquito 94 | Mosquito 95 | Mosquito 96 | Mosquito 97 | Mosquito 101 | Mosquito 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. | + | N.T. | N.T. | N.T. | + | + | + | + | + | N.T. | + | + | — | N.T. | N.T. |
| — | N.T. | + | + | — | — | + | + | ? | ? | ? | + | + | — | + | + | + | N.T. | D. | + | — | N.T | N.T. |
| + | + | + | ? | ? | ? | ? | ? | — | — | — | + | + | + | — | D. | — | — | .. | D. | D. | N.T. | N.T. |
| — | ? | — | — | + | — | — | — | N.T. | N.T. | N.T. | N.T. | — | — | — | .. | — | D. | .. | .. | .. | N.T. | N.T. |
| ?+ | + | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. | — | — | — | + | + | — | .. | — | .. | .. | .. | .. | N.T. | N.T. |
| — | N.T. | — | + | + | + | — | — | — | — | — | + | + | + | — | .. | — | .. | .. | .. | .. | N.T. | N.T. |
| N.T. | — | + | — | + | + | — | + | — | — | + | + | — | — | — | .. | + | .. | .. | .. | .. | N.T. | N.T. |
| — | + | + | + | + | — | + | + | — | + | + | + | — | + | — | .. | — | .. | .. | .. | .. | + | — |
| + | — | + | — | + | ?+ | + | — | + | M. | — | — | + | — | — | .. | — | .. | .. | .. | .. | Discontinued | Discontinued |
| — | D. | — | — | — | — | ?+ | — | + | M. | — | + | + | + | D. | .. | — | .. | .. | .. | .. | | |
| + | .. | + | M. | — | + | — | + | — | D. | D. | ?+ | + | + | .. | .. | + | .. | .. | .. | .. | | |

*Series*

| No. of Animal | Days | Mosquito 64 | Mosquito 65 | Mosquito 66 | Mosquito 67 | Mosquito 68 | Mosquito 69 | Mosquito 70 | Mosquito 71 | Mosquito 72 | Mosquito 73 | Mosquito 74 | Mosquito 75 | Mosquito 76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. 2242 | 12th day | — | N. R. | .. | — | — | D. | — | — | .. | N.R. | N.R. | N.R. | N.R. |
| | 13th day | — | — | .. | — | — | .. | — | — | .. | — | + | — | + |
| | 14th day | — | + | .. | — | — | .. | — | — | .. | D. | — | — | + |
| | 15th day | + | — | .. | — | D. | .. | D. | D. | .. | .. | — | — | M. |
| | 16th day | + | + | .. | — | .. | .. | .. | .. | .. | .. | + | — | .. |
| | 17th day | + | + | .. | — | .. | .. | .. | .. | .. | .. | — | — | .. |
| | 18th day | ?+ | — | .. | — | .. | .. | .. | .. | .. | .. | — | M. | .. |
| | 19th day | — | — | .. | — | .. | .. | .. | .. | .. | .. | — | D. | .. |
| | 20th day | N.R. | N.R. | .. | N.R. | .. | .. | .. | .. | .. | .. | M. | .. | .. |
| | 21st day | + | — | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 22nd day | — | — | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 23rd day | — | + | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 24th day | — | + | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 25th day | + | + | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 26th day | + | — | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 27th day | — | — | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 28th day | — | — | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| No. 2631 | 29th day | D. | D. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 30th day | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 31st day | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 32nd day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 33rd day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 34th day | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 35th day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 36th day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 37th day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 38th day | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| | 39th day | .. | .. | .. | D. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

"N.R." signifies "result
"N.T." signifies "not
"M" " that
"D." "
"E." "

*II.* (2)—contd.

| Mosquito 77 | Mosquito 77 (a) | Mosquito 78 | Mosquito 79 | Mosquito 80 | Mosquito 81 | Mosquito 82 | Mosquito 83 | Mosquito 84 | Mosquito 85 | Mosquito 86 | Mosquito 87 | Mosquito 88 | Mosquito 89 | Mosquito 90 | Mosquito 91 | Mosquito 92 | Mosquito 94 | Mosquito 95 | Mosquito 96 | Mosquito 97 | Mosquito 101 | Mosquito 102 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| — | .. | ? | M. | ? | ? | ? | ? | — | .. | .. | ?+ | — | + | .. | .. | — | .. | .. | .. | .. | .. | .. |
| + | .. | — | D. | — | — | — | — | + | .. | .. | — | + | + | .. | .. | N.T. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | + | + | — | — | — | .. | .. | + | + | + | .. | .. | M. | .. | .. | .. | .. | .. | .. |
| — | .. | — | .. | + | + | + | + | — | .. | .. | + | + | — | .. | .. | D. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | — | + | + | + | — | .. | .. | ?+ | — | D. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | + | .. | — | — | — | + | — | .. | .. | — | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | — | .. | M. | — | M. | — | + | .. | .. | + | D. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | D. | + | D. | D. | — | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | .. | + | .. | .. | + | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | + | .. | .. | + | .. | .. | + | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | — | .. | .. | — | .. | .. | — | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | .. | — | .. | .. | + | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | .. | + | .. | .. | + | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | + | .. | .. | + | .. | .. | — | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | .. | + | .. | .. | — | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | .. | — | .. | .. | M. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | .. | + | .. | .. | D. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | .. | + | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | .. | — | .. | .. | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | + | .. | .. | E. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| + | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | D. | .. | .. | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| — | .. | .. | •• | .. | .. | .. | .. | .. | .. | .. | — | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| D. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | + | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | D. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

not recorded."
tried."
the mosquito was moribund
" " " dead.
" " " escaped.

*The feeding experiments on rabbits.*

*History of mosquitos that were fed on rabbits.*

Mosquito 1. 27-VIII, 3 P.M. Emerged. Enclosed with raisin.
29.-VIII, 4 P.M. Removed raisin.
30-VIII, 3-4 P.M. Tried on two Two-day-strain Rabbits (on ears); mosquito punctured, but did not draw blood.

Mosquito 2. 28-VIII, noon. Emerged. Enclosed with raisin.
29-VIII, 4 P.M. Removed raisin.
30-VIII, 3-4 P.M. Tried on ears of two Two-day-strain Rabbits; mosquito punctured, but did not draw blood.

Mosquitos 3—10. 29-VIII. Emerged. Enclosed singly in tubes, with raisins.
30-VIII. Removed raisins.

Mosquitos 11—21. 30-VIII. Emerged. Enclosed singly in tubes, with raisins.
31-VIII. Removed raisins.

Mosquitos 22—27. 31-VIII. Emerged. Enclosed singly in tubes, with raisins.
1-IX, afternoon. Removed raisins.

Mosquitos 28—30. 1-IX. Emerged. Enclosed with raisins singly in tubes.
2-IX. Removed raisins.

Mosquitos 1—22. 1-X, 10-30 A.M.-2-30 P.M. Tried on Two-day-strain Rabbit 1995. No. 16 bit, but was not fully gorged; No. 22 did not bite; all the rest, except No. 6 (which was dead), were fully gorged.

Mosquitos 22—30. 3-IX. Tried on Two-day-strain Rabbit 2009 (2010 ?) (rabbit moribund); none bit.

Mosquitos 22—30. 3-IX, 11 A.M.-1 30 P.M. Tried on Two-day-strain Rabbit 1995; all were fully gorged.

The results of the feeding experiments are tabulated. The table shows the behaviour of each mosquito during feeding from day to day.

"a" indicates that the abdomen of the mosquito was already distended; "f" indicates that the mosquito bit, but was not fully gorged; "ff" indicates that the mosquito took a full feed; "i" represents that the mosquito was tried on the animal at intervals; "n" signifies that the mosquito was tried, but did not bite; "p" indicates that the mosquito punctured but did not draw blood; "r" signifies that that mosquito bit readily.

The feeding on normal rabbits was commenced on 3-IX. and terminated on 8-X. After that date the surviving mosquitos were tried on bulls, on account of the high mortality amongst the experimental rabbits, caused by hæmorrhagic septicæmia.

**Table showing biting propensities of A. (S.) albopicta.**

*Table showing biting propen*

| Rabbit or Bull No. | Date | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 | No. 8 | No. 9 | No. 10 | No. 11 | No. 12 | No. 13 | No. 14 | No. 15 | No. 16 | No. 17 | No. 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit 2042. | 3 | f | ff | ff | Killed | f | Dead | ff | f | ff | n a | ff | ff | ff a | n a | ff | ff | ff | f a |
| | 4 | Not tried | | | | .. | | Not tried | | | ff | Not tried | | | ff | Not tried | | | |
| | 5 | ff | ff | f | | ff | | ff | ff | ff | n a | ff | ff a | ff | n a | n | f a | ff | ff |
| | 6 | n a | n a | n a | | ff | | n a | n a | n a | fp a | n a | ff a | f a | ff | n | n | n | n a |
| | 7 | ff | n | ff | | f j | | ff | ff | n | ff | ff | ff | ff i | ff | | ff | ff | n |
| Rabbit 2043. | 8 | n | ffr | np | | ff | | fi | n | ff r | ffr | n p | ff a | n | [n a | | np | np | f i |
| | 9 | ffr | ffr | | | ffr | | ff r | ffr | n i | n ai | ffr | ffr a | ff | ff | | ffr | f | n i |
| | 10 | ffr | ffr | | | fla i | | ffr a | ff | n l | ffr | n a | ff a | ffr | f a | | ff i | ff | l |
| | 11 | n a i | n a i | | | n i | | ff r | n a i | f | ff a i | n a i | ff a | ff a | n i | | n a i | n | ffr |
| | 12 | ff i | n a i | | | f | | ff r | ff | fi r | f a | ff a i | ffr a | f | fi | | ffr | ffr | nl |
| Rabbit 2210. | 13 | n a i | n a i | | | f | | ff i | n | ff r | ff i | np a | ffr a | nl | ff | | ff a i | ff i | n i |
| | 14 | n i | ff i | | | ff | | n i | ffr | n i | fi | n i | ff | fi | n i | | ffr a | n i | n i |
| | 15 | n l | ffr | | | | | ff r | fr a | n i | n a i | n i | na i | ffr | fl | | ff m | ff i | fl |
| | 16 | ff | ff | Dead | | | | np a i | n i | np i | np a i | ff | ffr | n i | n i | Dead | ffr | n i | ffr |
| | 17 | n i | f a | | | | | f i | ffr | f | n i | n a i | n a i | n i | fi r | | np i | f | |
| Rabbit 2301. | 18 | ffi a | ffj | | | | | ff i | n a i | n i | f | n i | n i | n i | n i | | ff i | ff | |
| | 19 | n i | ff a i | | | | | ff i | n i | ffi | ffr | n i | ff | f | ffr | | np i | n i | |
| | 20 | n i | n i | | | | | np i | ff | n i | n i | n i | n a i | | n a i | | f | f i | |
| | 21 | np i | np i | | | | | np i | ff | n i | np i | n i | ff a | | n i | | ff | n i | |
| | 22 | np i | ff i | | | | | ff | n i | ff i | ff | ff i | ff a | | n i | | f i | ff i | Dead |
| Rabbit 2312. | 23 | fr | ff i | | | | | n a i | n i | fr | np a i | n a i | ffp a i | Dead | ffr | | n i | ff i | |
| | 24 | n i | ffr a | | | | | ffr a | fr | ffi | ff | n a i | np a i | | | | f | ff i | |
| | 25 | np i | np l | | | | | ff a | ff l | n i | ff a | n i | ff i | | Dead | | fi | fi | |
| | 26 | fr | | Dead | | | | ffa i | ffr | n f | n a i | ff | ffr | | | | ffi | nl | |
| | 27 | n i | | | | | | ff a | f i | ff | n i | n i | f a | | | | f | Dead | |

*N.B.* When "P" occurs with "f" or "ff," it indicates that for a while the mosquito punctured the

*sities of* A. (S.) albopicta.

| No. 19 | No. 20 | No. 21 | No. 22 | No. 23 | No. 24 | No. 25 | No. 26 | No. 27 | No. 28 | No. 29 | No. 30 | Time of experiment and condition of weather |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n a | n a | ff | Not tried | | | | | | | | | 2—5 P.M. |
| ff | ff a | Not tried | | | | | | | | | | 2-30—3 P.M. |
| ff | n a | ff | ff | ff | ff | ff | ff | ff | ff | ff n | ff | 10-30 A.M.—2-30 P.M. |
| n a | ff | ff a | ff a | n a | n a | n a | n a | np a | np a | n | np a | 11-45 A.M.—2-20 P.M. |
| ff i | n | n | n | n | f r i | ff | n | ff | ff | fr | ffr | 10-20 A.M.—1-20 P.M. |
| ffi | ffr | ffr | ff | n i | ffr | n a | ffr | np a | n a | ff i | f | 11 A.M.—1-30 P.M. |
| n a | n a i | ff | f | ffi | ffr | ffr a | ffr a | ffr | ff | f | ffr | 10-50 A.M.—1 P.M. |
| ff a | ffr | n a i | ff | n a i | ff | ff a | n a | ffr | ff | ffr | ff i | 10-50 A.M.—1-45 P.M. |
| ff a i | n a i | ffr | f a i | ffi | n a i | n a i | n i | ffr a | ff a | ff a i | f a | 11 A.M.—2 P.M. |
| ffr | np a | n i | ff i | n a | ffr | ffr | ff i | n a | n a | ff | n i | 2-35—4-50 P.M. |
| n i | n a i | f | np a | ff | np a i | ffr | ff | ffr | fr a | n i | ff | 1-25—3-25 P.M. |
| ff | ffr | ff | fi | n i | ffi | n a i | np a i | n a i | ff a | ff i | ffr | 1—3-30 P.M. |
| np a i | n a i | np i | np i | np i | ffr a | ffr a | n pai | n a i | np a i | np a i | ffr a | 3-30—5-15 P.M. |
| ff a | n i | Dead | ffp i | ff | ffr | np a i | ff | np i | ffr | ffi | n a i | 1-30—3 P.M. |
| n i | ffr | .. | n i | Dead | ffr a | np i | n a i | Dead | np a i | f i | np i | 1-20—3-11 P.M. |
| ffr | n i | .. | n i | .. | ffa | Dead | n i | .. | ffp a i | n i | n p a i | 2-30—4-21 P.M. |
| n i | n i | .. | ffr | .. | ffr a | .. | ffr | .. | ffr n | fr | ffr | 2-30—3-50 P.M. |
| fr | fr | .. | ff i | .. | np a i | .. | n a i | .. | ffp a i | n i | ff | 1-30—3-7 P.M. |
| fi | n a i | .. | np i | | ffr | .. | ffi | .. | ffr | n i | ffr | 12-15—1-50 P.M. |
| n i | n i | .. | n i | .. | np a i | .. | n a i | .. | ff | np i | ff | 2-30—4-15 P.M. |
| n i | ffr | .. | np i | .. | n a i | .. | n i | .. | np a i | np i | n i | 11-30 A.M.—1-21 P.M. |
| f | n i | .. | ffi | .. | np a i | .. | n i | .. | ff r | Dead | np i | 1-36—3 P.M. |
| Dead | ff | .. | n i | .. | fr | .. | f | .. | ffr | .. | n i | 1-39—3-24 P.M. |
| | Dead | .. | n i | .. | Dead | .. | Dead | .. | fa | .. | ff a i | 2-58—4 P.M. |
| .. | .. | .. | fi | .. | .. | .. | .. | .. | ff a | .. | ff a | 2-52—3-55 P.M. |

skin without drawing blood, but later on took a feed.

*Table showing biting propen*

| Rabbit or Bull No. | Date | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | No. 7 | No. 8 | No. 9 | No. 10 | No. 11 | No. 12 | No. 13 | No. 14 | No. 15 | No. 16 | No. 17. | No. 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit 2425. | 28 | ff i | | | | | | n i | n i | | ffr | n i | ff | | | | f | | |
| | 29 | ff a | | | | | | ff | ffr | | f | n i | ffr a | | | | n i | | |
| | 30 | n i | | | | | | f a | ff | | fa | | ff | | | | np i | | |
| | 1 | n i | | | | | | n i | n i | | np a i | | np a i | | | | n i | | |
| Rabbit 2448. | 2 | n i | | | | | | n i | ff | | ff | | ff | | | | n i | | |
| | 3 | ff r | | | | | | ff r | ff i | | f | | ff a | | | | n i | | |
| | 4 | ff i | | | | | | ffi | ff i | | np i | | ff a | | | | | | |
| | 5 | n i | | | | | | ffi | f a | | fp i | | f a | | | | | | |
| | 6 | ff | | | | | | n i | ff i | | ffr | | np a i | | | | | | |
| Rabbit 2527. | 7 | n i | | | | | | ff | n i | | np a i | | f a | | | | | | |
| | 8 | n i | | | | | | f a i | ff | | n i | | n | | | | | | |
| | 9 | | | | | | | Not tried | Not tried | | Not tried | | Not tried | Dead | Dead | Dead | | | |
| | 10 | | | Dead | Dead | Killed | Dead | | n i | Dead | n i | Dead | n i | | | | Dead | Dead | Dead |
| | 11 | | | | | | | | n i | | | | ff | | | | | | |
| | 12 | | | | | | | | | | | | ffr | | | | | | |
| | 13 | | | | | | | | | | | | n a i | | | | | | |
| | 14 | | | | | | | | | | | | ffr | | | | | | |
| Bull 2631 (Chupper 54). | 15 | Dead | | | | | | Dead | Dead | | Dead | | Not tried | | | | | | |
| | 16 | | | | | | | | | | | | n i | | | | | | |
| | 17 | | | | | | | | | | | | n i | | | | | | |
| | 18 | | | | | | | | | | | | np i | | | | | | |
| | 19 | | | | | | | | | | | | | | | | | | |
| | 20 | | | | | | | | | | | | | | | | | | |
| | 21 | | | | | | | | | | | | Dead | | | | | | |
| | 22 | | | | | | | | | | | | | | | | | | |

*N.B.* When "p" occurs with "f" or "ff," it indicates that for a while the mosquito punctured the

*sities of* A. (S.) albopicta—contd.

| No. 19 | No. 20 | No. 21 | No. 22 | No. 23 | No. 24 | No. 25 | No. 26 | No. 27 | No. 28 | No. 29 | No. 30 | Time of experiment and condition of weather |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| .. | .. | .. | n i | .. | .. | .. | .. | .. | ff | .. | ffr | 2-10 —2-53 P.M. |
| .. | .. | .. | Dead | .. | .. | .. | .. | .. | ff | .. | fa | 1-55—2-25 P.M |
| | | | | | | | | | n i | | ff | 12-45—1-40 P.M. |
| | | | | | | | | | f | | n i | 12-45 P.M. — not noted. |
| | | | | | | | | | ff | | n i | 2-25—3 P.M. (Cloudy and chilly.) |
| | | | | | | | | | ffr | | ff | 12-54 —1-20 P.M. (Uncertain weather.) |
| | | | | | | | | | np a i | | n i | 2—2-30 P.M. (Very chilly and sunny). |
| | | | | | | | | | n i | | n i | 1-8—1-40 P.M. (Uncertain weather.) |
| | | | | | | | | | ffr | | ffr | 12-20—12-54 P.M. (Uncertain weather.) |
| | | | | | | | | | ffr | | n i | 2-35—3-9 P.M. (Not chilly ; uncertain weather.) |
| | | | | | | | | | ffi | | ffr | 1—1-34 P.M. (Chilly and sunny.) |
| | | | | | | | | | Not tried | | Not tried | |
| | | | | | | | | | n i | | np i | 10-35—11-15 A.M. |
| | | | | | | | | | ffr | | ff i | 10-36—11-20 A.M. (Uncertain weather.) |
| | | | | | | | | | np a i | | ff r i | 10-45—11-22 A.M. (Sunny and chilly.) |
| | | | | | | | | | ffa | | n i | 10-15—10-45 A.M. (Sunny and chilly.) |
| | | | | | | | | | n i | | np a i | 10—10-42 A.M. (Sunny and chilly.) |
| | | | | | | | | | Not tried | | Not tried | |
| | | | | | | | | | n i | | n i | |
| | | | | | | | | | Dead | | n i | 9-40—10 A.M. (Uncertain weather.) |
| | | | | | | | | | | | ff | 10-10—10-50 A.M. (Sunny and chilly.) |
| | | | | | | | | | | | n i | 9-30—9-45 A.M. (Very chilly and sunny.) |
| | | | | | | | | | | | n i | 9-50—10-45 A.M. (Chilly and sunny.) |
| | | | | | | | | | | | n i Dead | 9-25 A.M (Chilly and sunny.) |

skin without drawing blood, but later on took a feed.

Remarks.

7-IX. No. 15 moribund at 11-30 A.M. and found dead at noon.
No. 16 bit when repeated attempts had been made to make it do so ; No. 18 without one leg ; No. 26 covered over with brown excrement. No. 1 undersized.

8-IX. No. 18 unable to poise and bit after repeated attempts.

9-IX. No. 13 took a long time to feed ; No. 14 punctured several times in attempting to feed ; No. 18 moves with a jerky motion ; No. 9 without one leg ; No. 28 fed in several instalments.

10-IX. No. 12 fed in instalments and showed marked tendency to fly towards muslin, although the latter was away from light ; No. 18 fed very slightly ; No. 9 sluggish.

11-IX. No. 9 fed slightly after several attempts ; No. 18 moved proboscis along margin of tube (although itself seated on animal) as if in search of something ; No. 1 sluggish ; No. 22 fed very slowly ; No. 5 undersized.

12-IX. No. 13 had abdomen very thin and took a long time to feed ; No. 11 fed when tried a third time ; No. 14 fed when tried a third time.

13-IX. No. 9 slightly injured ; No. 14 took a long time to feed ; No. 18 very weak ; No. 25 kept oscillating its body while feeding.

14-IX. No. 7 without one leg ; No. 20 moved proboscis like No. 18 on 11-IX ; No. 9 weak ; No. 13 fed slightly ; No. 21 took a long time to feed.

15-IX. No. 8 fed slightly ; No. 1 thin ; No. 9 thin ; No. 18 fed, although unable to balance itself.

16-IX. No. 11 fed in two efforts ; No. 13 probably slightly fed ; No. 18 behaved as it did on 11-IX ; No. 23 moribund(?); No. 8 made repeated efforts to bite.

17-IX. No. 13 moribund ; No. 17 fed slightly.

18-IX. No. 17 sluggish, but bit all on a sudden ; No. 24 would probably have fed at once, but rabbit difficult to immobilize; No. 25 moribund (3-20 P.M.); No. 24 imbibed only a small quantity of blood during first bite, but was fully gorged when bit a second time ; No. 25 dead at 4-30 P.M.

19-IX. No. 10 behaved like No. 18 on 11-IX ; No. 13 very sluggish and moved with difficulty, but energetically punctured skin, and collapsed all on a sudden, but revived again ; No. 14 fed in several efforts ; No. 28 fed in three efforts ; No. 9 took a long time to feed.

20-IX. No. 20 behaved as No. 18 did on 11-IX ; whether No. 24 had fed could not be definitely ascertained owing to insufficient light (cloudy day) ; No. 30 bit as the result of considerable teasing ; No. 19 fed when repeated attempts had been made to make it do so.

21-IX. No. 9 sluggish ; No. 19 suddenly took to feeding as the result of considerable teasing ; No. 26 looked as if stupefied while biting.

22-IX. No. 12, which fed in two instalments, took a fairly full feed during first bite and fed only slightly during second bite.

23-IX. No. 9 took a long time to feed ; No. 14 looked moribund, but bit (it behaved as No. 18 did on 11-IX) ; during first trial No. 17 looked as if it would feed, but never actually punctured skin ; No. 19 probably would have fed, but rabbit difficult to immobilize ; No. 12 fed readily when tried a second time ; No. 28 stabbed skin every time it was tried ; No. 29 looked moribund when tried a second time.

25-IX. Nos. 16 and 24 fed slightly.

26-IX. No. 1 fed slightly ; No. 20 moribund (1-30 P.M.) ; No. 7 fed in instalments ; No. 30 fed very readily when tried a second time ; No. 2 dead at 4—15 P.M. Examined contents of alimentary tract of No. 2 and noticed fresh blood in the anterior portion of stomach.

28-IX. No. 16 took a long time to feed ; No. 22 moribund and made efforts to puncture skin but failed ; No. 28 fed in repeated efforts and excreted frequently ; No. 22 dead at 2—53 P.M.

29-IX. No. 28 fed on repeated pressure.

30-IX. No. 28 fed very slightly (?). Rabbit moribund at 1-48 P.M.

1-X. Rabbit dead at 2 P.M.

3-X. Nos. 1 and 10 took a long time to feed ; No. 30 took a rather long time to feed ; No. 28 bit as the result of much pressure ; No. 16 moribund.

4-X. Several of the mosquitos (*e.g.*, Nos. 1 and 8), although they stubbornly refused to bite, bit as soon as the window was opened to let in chilly wind.

5-X. Nos. 7 and 10 bit when repeated efforts had been made to make them do so.

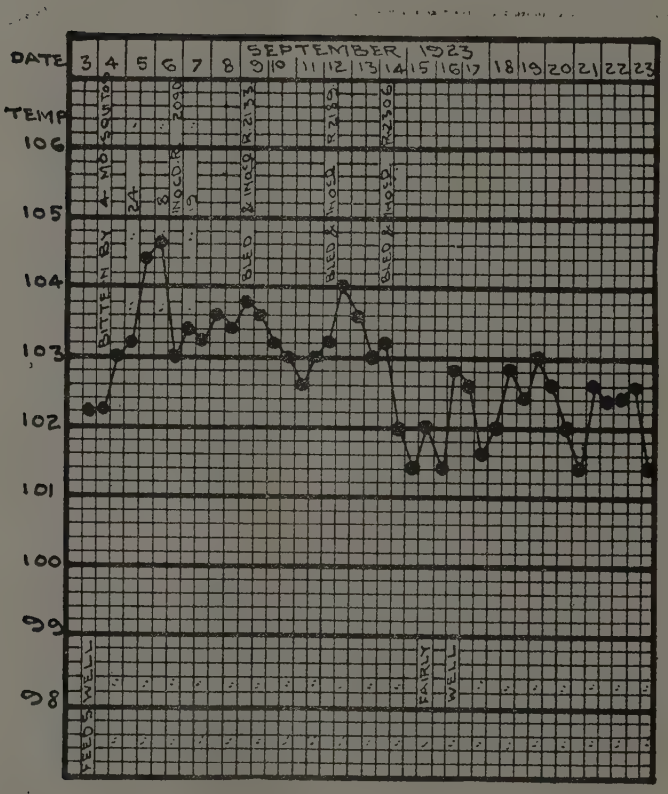
6-X. No. 12 punctured very readily when tried a second time.

7-X. No. 28 took a long time to feed.

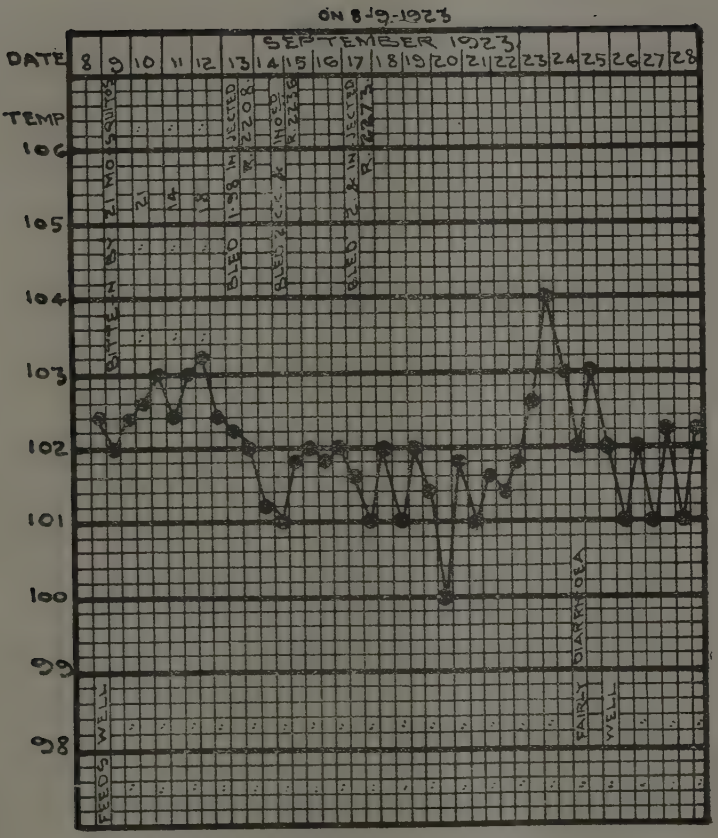
8-X. No. 7 fed slightly.

As will be seen from the foregoing table, there were eight rabbits bitten by infected mosquitos, of which two died during the progress of the experiments. The blood from the remaining six was " passaged " through a series of rabbits with a view to intensifying the thermal reactions manifested by the originally infected (bitten) rabbits. The blood was thus " passaged " through sixty-five rabbits divided into six series corresponding to the six rabbits bitten by infected mosquitos, but the interpretation of the results obtained by " passaging " was rendered difficult by the supervention of pasteurellosis amongst the experimental animals. During this period, the infectivity of particular strains of the rabbit virus was also tested upon cattle (nine bulls were thus used), but the results of these tests were negative.

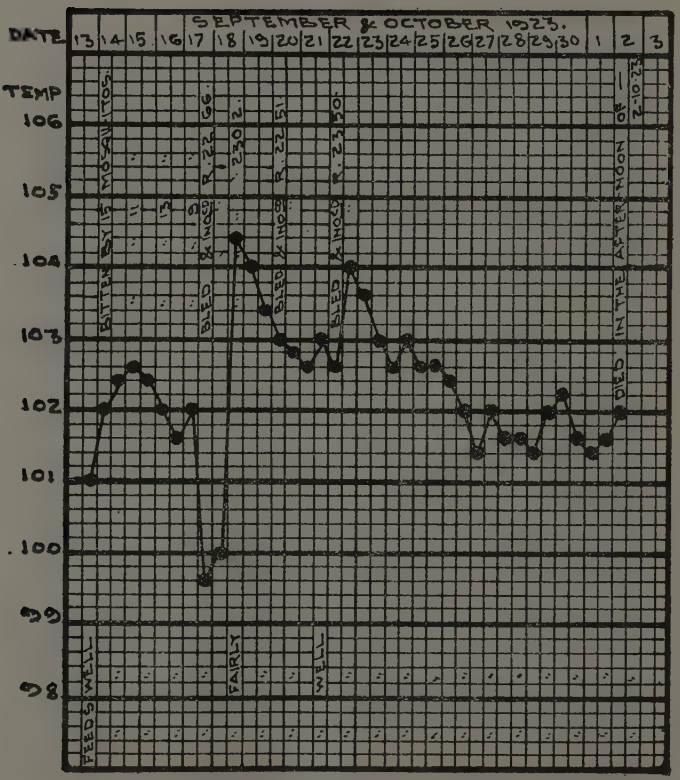
The clinical observations made on the six rabbits bitten by infected mosquitos are shown in the attached charts.



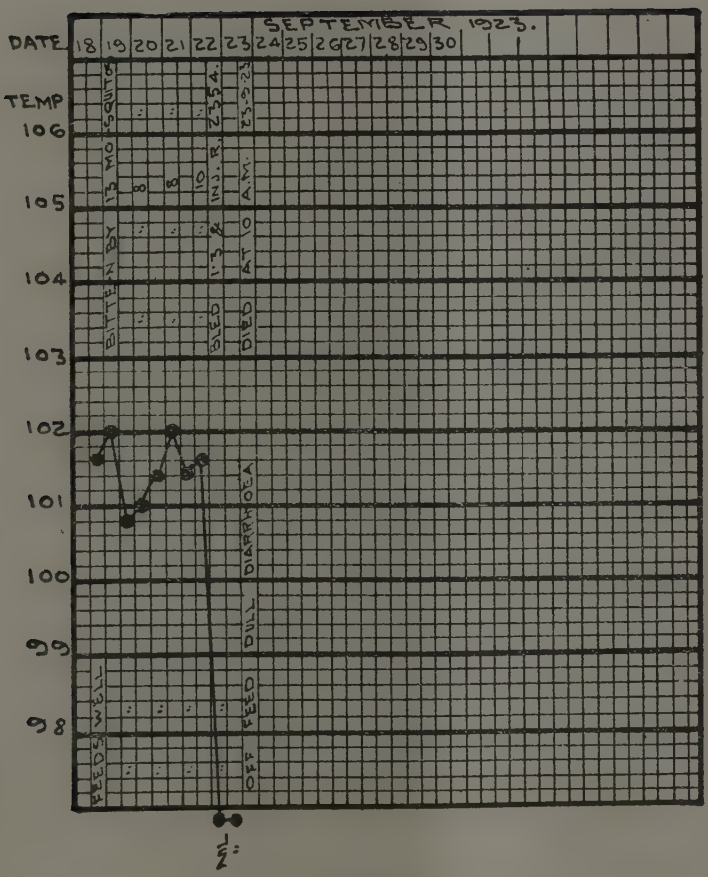

Rabbit 2042.
Bitten by 15 mosquitos (fed from R. 1995, 2 days' strain)
on 3—IX—1923.



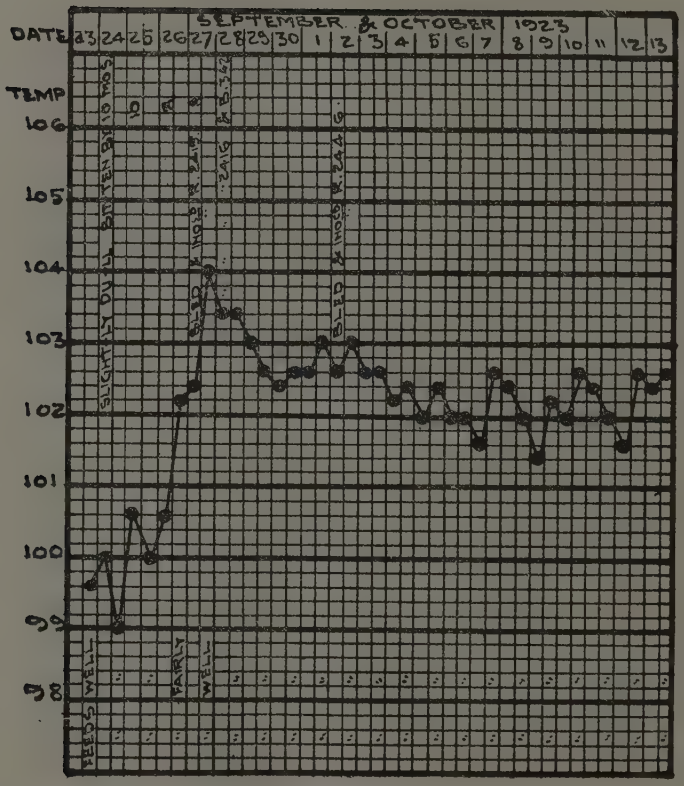

Rabbit 2043
Bitten by 15 mosquitos (fed from R. 1995, 2 days' strain)
on 8—IX—1923.



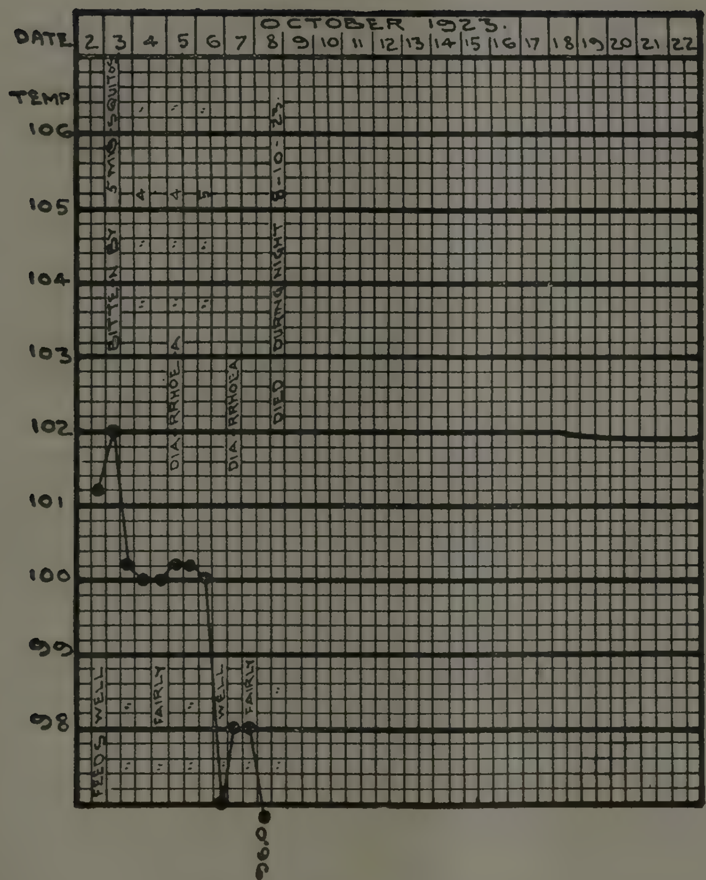

Rabbit 22101.
(Bitten by 15 mosquitos on 13—IX—1923.)




Rabbit 2301.
(Bitten by 9 mosquitos on 18—IX—1923.)




Rabbit 2312.
(Bitten by 7 mosquitos on 23—X—1923.)




Rabbit 2148.
(Bitten by 4 mosquitos on 2—X—1923.)

*The inoculation of the bodies of infected mosquitos into bulls. Result: Negative.*

1. Inserted Mosquito 50 (uncrushed) into Bull 2653, Chupper 46 ; 3-VII, 4-45 P.M.
2. Inserted Mosquito 51 (uncrushed) into Bull 2654, Chupper 44 ; 3-VIII, 4-15 P.M.
3. Inserted Mosquito 52 (uncrushed) into Bull 2661, Chupper 22 ; 3-VIII, 4-22 P.M.
4. Crushed Mosquitos 35, 42, 44 and 49 and inserted into Bull 2723, Chupper 38 ; 13-VIII, 5-45 P.M.
5. Crushed Mosquitos 53, 54 and 55 and inserted into Bull 2720, Chupper 34 ; 13-VIII, 6 P.M.

*The dissection of infected mosquitos.*

1. Fed one mosquito on Control 2350 on 29-VII, 3 P.M. Dissected and examined dark-ground on 29-VII, 5 P.M.

Corpuscles nearly intact, well-rounded, showing up brightly ; a small proportion had lost refractivity of edges. Around several corpuscles, which were apparently beginning to show distortion, there were festoons of short processes with blunted knob-like ends. The majority, however, appeared to be free, but in the relatively stationary parts of the preparation the number of festooned corpuscles appeared to be greater. In places there was evidence of the formation of fibrin in the form of scarcely visible, stationary, bristle-like threads. The plasma contained an enormous number of colloid particles, showing active Brownian movements ; more rarely, at the rate of 1 to 2 per field, there were larger active bright dots, which, on careful resolution, appeared to consist of 2 or 3 or more granules, joined together and performing active vibrionic movements. Sometimes long, thick, wriggling shreds could be seen, evidently pseudo-spirochætes, also bright coccoid particles. No forms could be seen definitely approaching *Leptospira*, although some of the forms, stated to be pseudo-spirochætes as a precautionary measure, displayed characteristics not far removed from those of *Leptospira*.

Examined salivary glands dark-ground. No motile structures but glandular cells and a number of stationary coccoid particles were seen.

2. *Mosquito 45.* Fed on Control 2410 on 28-VII, 12-30 P.M. Dissected and examined dark-ground on 29-VII, 5 P.M.

Aspirated some of the contents with salt solution. The resultant mixture was light bright-red in colour and appeared to contain minute coarse particles. Examined dark-ground, the red corpuscles were still seen to be intact, and also what appeared to be blood-platelets and some granular masses, probably leucocytes. Plasma contained an immense number of minute colloid particles, the great majority of which was stationary, but many executed Brownian movements. No definitely spirillar form was noticed. Occasionally faint corpuscles, that had lost refractivity were seen ; rarely some larger particles which had granular vibrionic form were

seen. In places the faint corpuscles appeared to be in a process of disintegration. Very rare pseudo-spirochætal forms were seen with prominent ends.

3. *Mosquito 48.* Fed on Control 2420 on 30-VII, 11-30 A.M. Dissected and examined dark-ground on 30-VII, 6-30 P.M.

Contained a few remnants of corpuscles, many still rounded, but not showing bright refractive edges. Very numerous colloid particles in the plasma, showing active Brownian movements, were seen ; a few showed granular vibrionic structure. Some extremely fine filaments were seen, probably pseudo-spirochætes, but occasionally one rather resembling a *Leptospira* was seen, with minute special structure. On close search many small structures of this kind were seen. Forms rather resembling Leishman's "granule clumps" could occasionally be seen represented by several bright granules within a delicate vesicular envelope. In fact, structures of this kind were very numerous when the preparation got to rest. Definite pseudo-spirochætes, actively lashing and attached to remnants of corpuscles or platelets were also seen. Some very long forms of this kind were also noticed.[1]

[1] The dark-ground examination of these three slides was kindly made by the Director of the Muktesar Laboratory.

## APPENDIX I.

### *The Life-history of* A. (S.) albopicta *in High Altitudes.*

### 1. *Mosquitos bred from Pusa tree-hole materials.*

*Pusa tree-hole materials I.*

(1)

11-V, 4-15 P.M. Put in water.
12-V, noon. Many found hatched.
20-V, 10 A.M. None pupated yet.
21-V, 10 A.M. Several pupated.
25-V, 10 A.M. No mosquito emerged yet.
26-V, 10 A.M. About 10 males emerged.
27-V. 15 males emerged.
28-V, 4 males emerged.
30-V. Only females emerging.

(2)

18-V. Put in water.
19-V. Found hatched.
29-V. Pupated.
31-V. No mosquito emerged yet.
1-VI. A large number of males emerged.
2-VI. A large number of males and 1 female only emerged.

(3)

31-V, 11-30 A.M. Put in water.
1-VI. Found hatched.
6-VI. Pupated.
9-VI. A large number of females and males (a larger proportion of the latter) emerged.

(4)

10-VI, 3-30 P.M. Put in water.
11-VI ,11-30 A.M. Found hatched.
18-VI. Both males and females emerged.

(5)

27-VI, 11-30 A.M. Put in water.
28-VI, morning. Found hatched.
4-VII. 1 only pupated.
5-VII. Many pupated.
8-VII. 4 or 5 males emerged.
9-VII. Several males emerged.
10-VII. 1 female and several males emerged.

*Pusa tree-hole materials I.*

11-VII, 2 P.M. 3 females emerged.
12-VII. 4 females emerged.
13-VII. 2 females emerged. Only 1 pupa left
15-VII. No further emergence yet.
16-VII. 1 male *A.* (*S.*) *thomsoni* emerged.

(6)

12-VII, 3-45 P.M. Put in water.
13-VII, 11 A.M. Found hatched.
21-VII, 2-30 P.M. A few pupated.
24-VII. Mostly pupated.
25-VII, 2 P.M. All not yet pupated. 1 male emerged.
26-VII, noon. About 10 males and 1 female emerged.
27-VII, 3 P.M. About 8 females and 2 males emerged.
28-VII, 11 A.M. 1 female emerged.
29-VII. None emerged.
30-VII to 1-VIII. Ditto.
2-VIII, 8 P.M. 1 female and 2 male *thomsoni* emerged.
3-VIII, noon. 2 male *thomsoni* emerged.

(7)

16-VII, 2-45 P.M. Put one handful of materials in water.
17-VII, noon. Found hatched.
26-VII, noon. A few pupated.
27-VII, 3 P.M. Many pupated.
1-VIII. 6 females and 2 males emerged
2-VIII, 4-20 P.M. 3 females and 1 male emerged.

(8)

2-VIII, 3 P.M. Put a large quantity of materials in water.
3-VIII, 12-50 P.M. Many found hatched.
11-VIII. Many young larvæ dead. Water turbid and stinking.

14-VIII t found pupated.
19-VIII, 7-30 A.M. 1 female emerged.
23-VIII, 11 A.M. 1 male emerged.
24-VIII. 1 male emerged.
noon. 1 female *thomsoni* emerged.
27-VIII, 11-20 A.M. 1 female *thomsoni* and 1 dwarf male *albopicta* emerged.

*N.B.* The tree-hole materials used in this experiment contained a large quantity of wild dry fruits and pieces of wood, and this probably accounted for the turbidity and foul smell of the water. Later on, the foul water was thrown away and fresh clean water was used after washing the materials themselves as thoroughly as possible, but this was of no avail, as most of the larvæ were already dead.

(9)

15-VIII, 3 P.M. Put in water.
16-VIII, noon. Hatched.
17-VIII, 4-15 P.M. Put in incubator (28°C.).
21-VIII, noon. 4 pupated.
22-VIII, noon. Mostly pupated.
23-VIII, 1 P.M. 3 males and 1 female emerged.
23-VIII, 4 P.M. 1 male emerged.
24-VIII, 5 P.M. 2 females emerged.
25-VIII, 1 P.M. No emergence. 3 pupæ and 2 larvæ left.
26-VIII, 1 P.M. No emergence.
27-VIII, 4 P.M. 1 female *albopicta* and 1 female *thomsoni* emerged.
28-VIII, 12-35 P.M. 1 female *albopicta* emerged. 3 P.M. 1 pupa left.
29-VIII, 2-30 P.M. No emergence.
30-VIII, 2 P.M. 1 female *thomsoni* emerged.
31-VIII, 11 A.M. The newly-emerged *thomsoni* found dead.

(10)

21-IX, 3 P.M. Put in water.
3-45 P.M. Put in incubator (28°C.).
22-IX, 2-5 P.M. Hatched.
26-IX, 1-10 P.M. 1 pupated.
28-IX, 3 P.M. All pupated.
29-IX, 4-30 P.M. 2 females emerged.
30-IX, 1-56 P.M. No emergence.
1-X, 2 P.M. Only 1 male *thomsoni* emerged. No pupa left.
2-X, 3-20 P.M. The male *thomsoni* still alive.
4-X, 3 P.M. The male *thomsoni* found dead.

*Pusa tree-hole materials II.*

(1)

11-V, 4-20 P.M. Put in water.
12-V, noon. Hatched.
21-V, 10 A.M. None pupated yet.
22-V, 10 A.M. Ditto.
23-V, 10 A.M. Pupated.
26-V. No adult emerged yet.
27-V, 1 male emerged.
28-V. 2 males emerged.

(2)

18-V. Put in water.
19-V. Hatched.
27-V. Pupated. (Dates of emergence of adults not noted.)

(3)

13-VI, 3-30 P.M. Put in water.
14-VI, morning. Hatched.
20-VI. Pupated.
(Dates of emergence of adults not noted.)

*Pusa tree-hole materials II.*

(4)

9-VII, 4 P.M. Put a large quantity of materials in water.
10-VII, 2 P.M. None found hatched yet.
11-VII, 1 P.M. Hatched.
19-VII, 4 P.M. Mostly pupated.
21-VII, 2-40 P.M. Except one, all the larvæ pupated.
23-VII, 2 P.M. 1 female emerged.
24-VII, 11-45 A.M. 3 females emerged.
25-VII, 2-30 P.M. 1 female emerged.
2-VIII. 1 female *thomsoni* emerged.

(5)

2-VIII, 4 P.M. Put a large quantity of material in water.
3-VIII, 1 P.M. Hatched.
15-VIII, noon. 4 pupated.
16-VIII, noon. 3 more pupated.
18-VIII, 1 P.M. 1 male emerged.

19-VIII, 1 P.M. 2 males emerged.
20-VIII, noon. 2 males and 1 female emerged.
21-VIII, noon. 1 male and 1 female emerged.
22-VIII, noon. 1 female emerged. Only a few larvæ left.
23-VIII, 11 A.M. 2 pupæ and 1 larva in vessel.
25-VIII, noon. Ditto.
28-VIII, 3 P.M. Ditto.
1-IX, 4 P.M. Only one larva left.
5-IX, 3-15 P.M. Ditto.
12-IX, 12-12 P.M. The larva found pupated.
18-IX, 2 P.M. 1 female *thomsoni* emerged.

(6)

21-IX, 3 P.M. Put a fairly large quantity of materials in water.
22-IX, 2 P.M. None found hatched.
24-IX, 4 P.M. Ditto.
29-IX, 1-6 P.M. 2 slightly grown-up larvæ seen in vessel.
4-X, 3-26 P.M. Larvæ evidently not full-grown.
9-X, 2-45 P.M. Only 1 (evidently almost full-grown) larva seen in vessel. (Water very cold.)
11-X, 1-15 P.M. Larva not pupated yet.
13-X, 1-15 P.M. Ditto.
17-X, 12-15 P.M. Ditto.
21-X, 11-30 A.M. Larva in same condition. (Temperature of water 12·5°C.)
26-X, 4 P.M. Larva dead. (Temperature of water 17°C.)

### 2. *Mosquitos reared from eggs deposited in captivity.*

*N.B.* Except where otherwise mentioned, there was always a supply of sugar solution or raisins for the enclosed mosquitos to feed upon, and of moist filter paper to oviposit upon.

(1)

19-VI, 3 P.M. Enclosed several males and 3 females in bottle A—all bred under laboratory conditions but early history not noted.
22-VI, 3-30 P.M. Offered arm. 2 engorged and transferred to bottle C; the other did not bite.
27-VI, 11 A.M. 1 female in bottle C dead. Offered arm to the remaining mosquito in bottle A; bit.
28-VI, 3 P.M. The remaining mosquito in bottle C dead. 4-30 P.M. Transferred mosquito from bottle A to cloth cage into which a male was also introduced. Offered arm; bit.
29-VI, 11-30 A.M. Offered arm; did not bite. 4-15 P.M. Offered arm again; did not bite.
30-VI, 3 P.M. Offered arm; did not bite. Found pairing for a minute. 4 P.M. Found pairing again. Offered arm; bit.
2-VII, 4 P.M. Offered arm; bit. Introduced a newly-emerged male. Observed pairing.
3-VII, noon. Offered arm; did not bite. Added water to sugar solution.
4-VII, 3 P.M. Offered arm; bit. Both the males alive.
5-VII, 3-30 P.M. Offered arm; did not bite. Introduced fresh water and fresh sugar solution.

6-VII, 4 P.M. Offered arm ; did not bite. Males alive.
7-VII, 12-30 P.M. Offered arm ; did not bite. 1 male dead.
8 VII. Offered arm ; did not bite. Enclosed mosquito in tube and tried again on arm ; did not bite. Surviving male alive. Placed back mosquito into cloth cage.
9-VII, 12-30 P.M. Offered arm ; did not bite. Introduced more males.
10-VII, 2-20 P.M. Mosquito distended with sugar solution. Offered arm ; did not bite. Introduced 5 males.
11-VII, 3 P.M. Offered arm ; did not bite. Female sluggish. Males happy.
3-VII, 4-30 P.M. Female sluggish. Males alive. Offered arm ; did not bite. Introduced fresh sugar solution.
15-VII, 1 P.M. Offered arm ; did not bite.
16-VII, 4 P.M. 12 eggs found deposited (Batch 1). Female seated close to moist filter paper. Introduced fresh sugar solution. Offered arm ; did not bite. Female sluggish. Males alive.
17-VII, 12-15 P.M. Female distended and found sucking sugar solution.
18-VII, 3-30 P.M. Female slightly stuck up in the sugar solution and much distended. It lost one leg while being disengaged by means of a pointed rod. Introduced 6 males bred from Pusa tree-hole materials II.
19-VII, 4 P.M. No more eggs deposited. Mosquito found seated on wall of cage.
20-VII, 3 P.M. Offered arm ; did not bite.
21-VII, 3-30 P.M. Removed female to bottle. Offered arm ; did not bite. Mosquito distinctly gravid.
22-VII, 2-30 P.M. About 20 more eggs laid (Batch 2). Female moribund.
23-VII, 3-20 P.M. Some eggs of batch 1 found hatched.
24-VII, 11-50 A.M. Mosquito still alive. 3 P.M. Transferred larvæ of batch 1 to a mixture of Pusa tree-hole materials II and water, prepared on 9-VII.
25-VII, 2 P.M. Female still alive and seated on moist filter paper. Larvæ of batch 1 growing.
27-VII, 3-30 P.M. Female found dead. Eggs of batch 2 not yet hatched.
30-VII, 10 A.M. One or two eggs of batch 2 found hatched.
31-VII, 4 P.M. No more eggs of batch 2 appear to have hatched. Transferred both eggs and larvæ to a mixture of Pusa tree-hole materials II and water, prepared on 9-VII.
1-VIII, 2 P.M. Larvæ of batch 1 growing steadily. Many eggs of batch 2 hatched but larvæ of unequal size.
2-VIII, 3-30 P.M. All growing steadily.
4-VIII 2-20 P.M. All the larvæ of batch 1 found pupated (7 pupæ in all).
9-VIII 1-45 P.M 3 males emerged from pupæ of batch 1.
10-VIII, 2 P.M 2 females and 1 male emerged from pupæ of batch 1. Kept these in bottle with raisins.

11-VIII, 4 P.M. Larvæ of batch 2 distinctly uncomfortable. Noticed a fungus-like deposit on water. Larvæ of unequal size. Removed the larvæ, one by one, to a cleaner mixture, prepared on 2-VIII, of Pusa tree hole materials II and water.

12-VIII, 2-30 P.M. Added 2 grains of rice to the water containing the larvæ of batch 2.

14-VIII noon. Transferred larvæ of batch 2 to clean tap-water in which a few grains of rice had been placed. 2 larvæ of batch 2 pupated. Tried the two femles of batch 1 (emerged on 10-VIII) on Control 2461 ; both bit (Mosquitos 56 and 57), one being seen pairing prior to feeding.

15-VIII, 11 A.M. One of the two pupæ of batch 2 dead. Added 3 dry specimens of *Musca* sp. to the water containing larvæ of Batch 2.

16-VIII, noon. 1 male emerged from batch 2.

*N. B.* The following observations relate to batch 2.

17-VIII noon. 1 pupated, but pupa very small in size.

20-VIII, noon. 1 more pupated.

21-VIII, 11 A.M. 2 more pupated. 1 female emerged.

22-VIII, 3 P.M. No emergence. Altogether 5 pupæ now.

25-VIII, 11-30 A.M. 1 female emerged. Except 2 larvæ, all pupated.

26-VIII, 11-45 A.M. 1 female emerged.

27-VIII, 11-20 A.M. Ditto.

2-30 A.M. Ditto.

28-VIII, 2-30 P.M. No further emergence.

29-VIII, 1 P.M. 1 male and 1 female emerged.

30-VIII, 1 P.M. No further emergence.

1-IX, 3 30 P.M. 2 females emerged.

(2)

20-VII, 3 P.M. Enclosed 4 three-day old males and 2 females. (One female emerged on 17-VII, the other on 18-VII, both from Pusa tree-hole materials II.) Offered arm ; both females bit.

21-VII, 3 P.M One male dead. Offered arm. 1 female bit readily; the other did not bite.

23-VII, 4 P.M. 1 male dead. Offered arm ; none bit. 1 female moribund (evidently the one which had refused to bite yesterday).

24 VII, 4 P M. The moribund female still alive; the other very sluggish. Both seated on moist filter paper and could not be induced to move.

25-VII, 2 P.M. Both females alive and still seated on moist filter paper. 1 male only alive.

27-VII, 3-30 P.M. 1 female dead ; the other laid 60 eggs and was moribund (having lost one leg).

28-VII, 11-30 A.M. The female alive. No more eggs laid.

29-VII, 10 A.M. The female dead. No more eggs laid.
31-VII. None of the eggs hatched.
1-VIII. Ditto.
2-VIII, 3 P.M. Several eggs hatched. Placed the newly hatched larvæ in a decoction of Pusa tree-hole materials II.
12-VIII, 1 P.M. Larvæ not growing satisfactorily.
13-VIII, 2 P.M. Added 2½ grains of rice to the water.
14-VIII. All the larvæ transferred to bottle (they had been so long in the lid of a bottle).
15-VIII, 11-30 A.M. Added 3 dry specimens of *Musca* sp. and many of the larvæ attacked them immediately although the specimens were floating.
17-VIII, 3-15 P.M. Larvæ growing steadily. Dry flies still floating. Smashed the flies by pressing them hard with forceps, when many of the pieces sank immediately (this was done in view of the fact that *Stegomyia* larvæ are bottom-feeders). A larva seen carrying about a large piece in its mouth, being afterwards joined by many more larvæ.
18-VIII, 1 P.M. Added 4 more dry flies.
21-VIII, noon. None pupated.
4 P.M. A few pupated ; pupæ medium-sized.
23-VIII, 11 A.M. Altogether a dozen larvæ pupated.
25-VIII, 11-30 A.M. The majority of the larvæ pupated.
26-VIII, 11-45 A.M. 1 male emerged.
27-VIII, noon. About 10 males and 1 female emerged. The remaining pupæ and larvæ transferred to a large bottle, with plenty of water.
28-VIII, 1 P.M. About 15 males and 8 females emerged. None of the mosquitos could be induced to leave surface of water apparently owing to low temperature.
4 P.M. All the larvæ pupated except three or four.
29-VIII, noon. 1 more female emerged. Failed to induce emerged mosquitos to leave surface of water ; only 2 females came up as the result of continuously shaking the bottle. The mosquitos refused to leave surface of water even when disturbed with a rod. Pupæ and larvæ also very sluggish. Temperature of room, 18°C.
2-4 P.M. 2 females came up as a result of bottle being kept in incubator for 15 minutes ; the rest had to be removed by means of a rod. Placed bottle in incubator.
30-VIII, 1-30 P.M. 13 females and 4 males emerged. Mosquitos quite active and very readily came up. All larvæ pupated by now.
31-VIII, 12-30 P.M. 6 females emerged. Emerged mosquitos quite active and very readily came up.
1-IX, 2-15 P.M. 3 females emerged and quite active.

*N. B.* All the mosquitos emerged in this series were used for feeding on rabbits.

(3)

26-VII. Introduced 1 newly-emerged female and 7 newly-emerged males into cloth cage.

27-VII. Added 2 more females and 3 more males.

28-VII, 3 P.M. Offered arm ; none bit, Transferred mosquitos to bottle.

30-VII, 10 A.M. 2 males dead.

noon. Removed raisins. Changed filter paper.

31-VII, 2-40 P.M. Offered arm ; all the 3 females bit readily. 1 more male dead. Put in a raisin.

2-VIII, 4-30 P.M. 1 male dead.

4-VIII, 3 P.M. Offered arm ; none bit. 1 male dead. Removed raisin as it had been covered over with fungus. Changed filter paper.

6-VIII, 3 P.M. Offered arm. 1 bit ; the rest did not bite, although they were several times induced to settle on the arm. 1 male dead and 1 moribund. Put in a raisin.

8-VIII, 4-20 P.M. Offered arm ; did not bite. Changed filter paper.

9-VIII, 3 P.M. Offered arm ; 1 bit. Put in a fresh raisin.

10-VIII, 2-30 P.M. Offered arm ; none bit. All the mosquitos distended with juice of raisin. 3 males alive. Removed raisin.

11-VIII, 3 P.M. Offered arm ; only 2 bit. 2 males alive. Put in a raisin.

12-VIII, 12-30 P.M. 3 eggs deposited on moist filter paper. Only 1 male alive. Removed raisin.

13-VIII, 2 P.M. About 80 eggs deposited on moist filter paper (Batch 1). All females and only 1 male alive.

14-VIII, noon. No more eggs deposited. 1 female (evidently the one that had oviposited) moribund ; its abdomen was open for more eggs, but none could be found.

15-VIII, 3-30 P.M. About 50 light-black and a few white eggs deposited on moist filter paper (Batch 2). 1 female (apparently the one that had oviposited) moribund. Offered arm to the remaining mosquitos ; bit. Removed the two batches of eggs, along with the filter paper on which they had been deposited, and supplied fresh moist filter paper to the remaining mosquito.

16-VIII, noon. The colour of the light-black eggs deepened to some extent.

17-VIII, 11-45 A.M. All the eggs, except the white ones, now turned black. No male alive.

18-VIII, 1 P.M. The eggs that were submerged were removed, one by one, to a moist piece of filter paper and kept exposed to atmosphere. This was done in view of the opinion of some observers, that submerged eggs do not hatch or take a long time to hatch.

19-VIII, 10-45 A.M. 2 eggs hatched.

20-VIII, 4-45 A.M. Many eggs hatched. The young larvæ and the unhatched eggs transferred to bottle, rice grains and dry flies being offered as food. About 80 eggs laid by the surviving mosquito (Batch 3).

*N. B.* The following observations relate to batches 1 and 2. The observations relating to batch 3 are given separately. Batches 1 and 2 were kept together.

25-VIII, 11-30 A.M. A few young larvæ seen to-day; these probably hatched out of eggs of batch 2.

29-VIII, 11-50 A.M. Larvæ growing steadily.

4-IX, 11-45 A.M. Several pupated.

5-IX. Pupæ and larvæ good-sized (*cf.* pupæ and larvæ of batch 3 in incubator).

7-IX, 4 P.M. 1 male emerged.

8-IX, 5 P.M. 2 males emerged.

9-IX, 2 P.M. 1 female and several males emerged. Adults good-sized.

10-IX, 3-30 P.M. 5 males and 1 female emerged. The newly-emerged adults could not be induced to leave surface of water.

4-30 P.M. bottle placed in incubator (28°C.).

11-IX, 3 P.M. 8 females and 5 males emerged.

12-IX, 5-20 P.M. 3 females emerged (all of good-size). 3 big larvæ and a few small larvæ now left.

13-IX, 2-45 P.M. 3 females emerged.

14-IX, 4 P.M. No further emergence.

15-IX, 4-20 P.M. 2 males emerged.

16-IX, 1-20 P.M. No further emergence.

17-IX, 4-30 P.M. 2 females emerged. 2 pupæ and a small larva left.

18-IX, 4-30 P.M. 1 female emerged.

24-IX, 4-10 P.M. 1 female emerged.

*Batch 3.* (See against date 20-VIII above.)

20-VIII, 11-45 A.M. About 80 eggs laid.

27-VIII, 11-50 A.M. None hatched yet.

28-VIII, 1 P.M. Many hatched.

2 P.M. The young larvæ and the unhatched eggs removed to a small bottle, 10 dry flies and 4 grains of rice being offered as food.

2-45 P.M. Placed bottle in incubator.

2-IX, 11-30 A.M. 2 larvæ pupated, but pupæ not of good size. Larvæ active, but thin.

noon. Removed larvæ and pupæ to a big bottle with plenty of food.

4-IX, 2-30 P.M. Several larvæ pupated. Larvæ distinctly better.

5-IX, 2-30 P.M 6 males emerged. Some more larvæ pupated, but still many unpupated.

6-IX, 10-30 A.M. Many males emerged.

7-IX 2-30 P.M. 3 females emerged.

8-IX, 2-30 P.M. 2 females emerged. Still a few larvæ unpupated.
9-IX, 1 P.M. 6 females emerged.
10-IX, 2-30 P.M. 7 females and 1 male emerged. All larvæ pupated.
11-IX, 2 P M. 5 females emerged.
12-IX, 5 P.M. 5 females emerged.

(4)

30-VII. Enclosed 4 newly-emerged males in bottle.
31-VII, 3-15 P.M. Introduced 3 females.
1-VIII, 2-15 P.M. Introduced 2 more males. 1 female accidentally injured. Introduced 1 newly-emerged female. Changed filter paper and raisins.
2-VIII, 4-30 P.M. Removed raisins. Introduced 1 newly emerged male.
4-VIII, 2-30 P.M. Offered arm ; all the 4 females bit.
6-VIII, 3 P.M. 1 male dead.
8-VIII, 4 P M. Offered arm ; 2 only bit ; 1 persistently refused to feed, whilst the fourth was moribund. Changed filter paper. Only 1 male alive.
9-VIII, 2 P.M. Only 1 male alive. Offered arm ; 1 bit. Introduced 3 newly-emerged males.
10-VIII, 1-30 P.M. Offered arm ; only 1 bit. Put in raisins.
11-VIII, 2-30 P.M Removed raisins.
12-VIII, 12-30 P.M. All the 4 males alive.
13-VIII, 2-30 P.M. 1 female dead. Put in raisins. Changed filter paper.
15-VIII, 2-45 P.M. A male and a female seen pairing seven times during about ten minutes. Offered arm ; 2 bit (the one that had paired did not bite).
16-VIII, 12-45 P.M. Offered arm ; 2 bit (including, apparently, the one that had paired yesterday).
17-VIII, 11-35 A.M. One female moribund, its body lying doubled up in water ; it could not be brought to normal position. Only 2 males alive.
18-VIII, noon. The moribund female of 17-VIII still responded when disturbed. Its abdomen, when squeezed for eggs, expressed only dark blood. 1 more female dead.
19-VIII, 10-30 A.M. The abdomen of mosquito that died on 18-VIII was squeezed for eggs ; only white undeveloped ova seen. Changed filter paper.
22-VIII, 11-30 A.M. The surviving female dead.

(5)

23-VIII, 4 P.M. Enclosed in bottle 1 newly-emerged female, 4 newly emerged males and 2 four-day old males. Bottle kept in incubator at 28°C. The newly-emerged mosquitos were from Pusa tree-hole materials I.
25-VIII, 1-15 P.M. Introduced 2 more females (emerged from Pusa tree-hole materials I). 1 male dead. Rasins covered over with fungus.
3-30 P.M. Changed raisins.

26-VIII, 1-10 P.M. No further mortality. A male and a female seen pairing.

27-VIII, 1 P.M. None dead. Tried on arm ; 2 females bit. Removed raisins as they were covered over with fungus. Washed filter paper to remove traces of raisin.

28-VIII, 3 P.M. Offered arm ; none bit.

29-VIII, 2 P.M. 1 male dead. Offered arm for a long time ; none bit. Mosquitos very sluggish when brought out of incubator for feeding. (Temperature of room, 18°C.)

30-VIII, 2 P.M. 30 eggs deposited on filter paper. No further mortality.

31-VIII, 11 A.M. 15 more eggs deposited. No mortality.

3 P.M. Offered arm for a long time ; only 1 female bit.

1-IX, 2-15 P.M. About 60 eggs (altogether) counted to-day. None of the eggs hatched. All males dead, but females all alive.

*N.B.* All the eggs laid between 30-VIII and 1-IX are included under batch I and further observations relating to these are given separately.

2-IX, 11-30 A.M. No mortality.

1-30 P.M. Offered arm ; none bit. Mosquitos sluggish as soon as brought out of incubator.

4-IX, 11 A.M. About 15 more eggs deposited. 1 female moribund (apparently the one that oviposited).

5-IX, 3 P.M. Altogether about 100 eggs counted to-day. 1 female dead.

6-IX, 11 A.M. 1 egg only hatched.

7-IX, 12-15 P.M. Many eggs hatched. All the mosquitos dead. Transferred larvæ to bottle containing water with dry flies and rice grains.

11-IX, 4-30 P.M. Larvæ of uniform size, but undeveloped. Many of the rice grains half-boiled (apparently owing to the heat of the incubator), and this imparted to the water a rancid smell. Separated the larvæ from the water by pouring the materials on a piece of muslin spread over the mouth of a big bottle. Washed the larvæ gently with tap-water and transferred them to clean water containing dry flies only.

15-IX, 1-20 P.M. Larvæ not growing uniformly.

19-IX, 4 P.M. 1 pupated (pupa very small). Larvæ not of uniform size, but quite happy.

20-IX, 3-50 P.M. 1 more pupated.

21-IX, 2 P.M. 2 or 3 more pupated.

22-IX, 2-10 P.M. Many pupated.

23-IX. No adult emerged yet.

24-IX. 4 males emerged.

25-IX, 5 P.M. 4 males and 1 female emerged.

26-IX, 5 P.M. 2 females emerged, both of very small size.

27 IX 4-13 P.M. 2 females and 1 male emerged, all of small size.

28-IX, 3 P.M. No emergence to-day. Still some in the larval stage, but there were a few good-sized pupæ.

29-IX, 4-50 P.M. 1 male and 1 female emerged; male dwarfish, female of medium size.

30-IX, 2 P.M. No emergence to-day. Several well-developed pupæ and many larvæ of unequal size.

1-X, 2 P.M. 2 dwarfish males and 1 female emerged

2-X, 3-5 P.M. 1 male emerged.

3-X, 1-40 P.M. 1 female emerged.

4-X, 2-45 P.M. No emergence.

5-X, 5 P.M. 1 male emerged.

6-X, 1 P.M. 2 males emerged.

7-X, 1-18 P.M. 1 male emerged.

2-7 P.M. As the larvæ were not growing well, a quantity of the water was thrown away and more dry flies and some rice grains were added.

8-X, 3 P.M. 1 male emerged.

9-X, 3 P.M. No emergence.

10-X, 1 P.M. 1 male and 1 female emerged. Rice grains fermenting and water smelling rancid.

11-X, 11-15 A.M. No emergence.

12-X. Ditto.

13-X, 3 P.M. 2 females emerged.

14-X, 11-30 A.M. No emergence.

15-X, 1-30 P.M. 4 females and 1 male emerged.

16-X, 1-30 P.M. 1 mosquito emerged (sex not noted).

17-X, 1-5 P.M. 2 females emerged.

18-X, 1 P.M. No emergence.

*Batch I.* (See against date 1-IX above.)

2-IX, 11-30 P.M. Some eggs hatched. Transferred the larvæ and the unhatched eggs to a big bottle containing plenty of water with dry flies and rice grains, kept in incubator at 28°C.

6-IX, 10-30 A.M. Added plenty of dry flies.

7-IX, 2 P.M. Larvæ not of uniform size.

10-IX, 3 P.M. 1 pupated.

11-IX, 3-45 P.M. 5 or 6 pupated. Larvæ not of uniform size.

13-IX, 3-40 P.M. 2 males emerged.

14-IX, 4P.M. 1 male and 1 good-sized female emerged.

15-IX,4-2 0 P.M. 2 females and 2 or 3 males emerged. One of the females could not be induced to leave the surface of water.

16-IX, 1-20 P.M. 4 females emerged.

17-IX, 4-30 P M. 7 females emerged.

18-IX, 5 P.M.. Only 1 male emerged. Several larvæ and some pupæ still left.

19-IX, 4-20 P.M. 2 males emerged.
20-IX, 3-45 P.M. 2 males and 1 female emerged.
21-IX, 2 P.M. 1 male emerged.
22-IX, 2-10 P.M. 1 female emerged.
23-IX, 1-30 P.M. 3 males and 1 female emerged.
24-IX, 4-20 P.M. 2 females and 1 male emerged.
25-IX, 5 P.M. 4 males emerged.
26-IX, 4-30 P.M. 3 females and 2 males emerged. Only 3 full-grown larvæ and no pupa left.
1-X, 2 P.M. 1 pupa, 1 apparently full-grown larva and 1 young larva seen.
2-X, 3-5 P.M. 1 female emerged.
3-X, 1-36 P.M. 1 full-grown larva and 1 young larva left.
4-X, 4-45 P.M. Ditto.
6-X, 1 P.M. Ditto.
7-X. Both larvæ dead.

(6)

29-IX, 4-48 P.M. Enclosed in bottle 2 females (emerged from Pusa tree-hole materials 1) and 1 small-sized male (bred from eggs deposited in captivity). Kept in incubator (28°C.).
1-X, 2-15 P.M. Introduced 2 more small-sized males. Changed raisins as they were covered over with fungus.
2-X, 3-15 P.M. Offered arm ; did not bite. Removed raisins.
4-X, 3-15 P.M. Offered arm ; did not bite, but 1 appeared attracted. (Temperature of room, 19°C.)
5-X, 4-30 P.M. Offered arm. 1 bit but was not fully gorged ; the other very sluggish and refused to bite even when made to settle on arm.
7-X, 1-19 P.M. 1 female seated on the filter paper.
8-X, 3 P.M. 1 only of the males alive.
11-X, 1-14 P.M. Male dead. Only 1 egg deposited.
12-X, 1-40 P.M. 2 more eggs found in water. 1 mosquito just deposited two clusters of eggs, one consisting of about 7 eggs, the other of 25 eggs, the former being deposited on the comparatively dry part of the filter paper, the other being stuck on to the leg of the mosquito. The mosquito did not respond when disturbed.
13-X, 3 P.M. Only 1 female alive.
14-X, 11-30 A.M. The female alive. No more eggs deposited.
16-X, 3-25 P.M. None of the eggs hatched.
18-X, 1 P.M. 1 larva seen ; it probably hatched on 17-X, as judged by its size.
19-X, 1-24 P.M. No further eggs hatched.
21-X 10-20 A.M. Many eggs hatched. Placed the young larvæ in water, previously used for rearing *albopicta* and which already contained dry flies, rice grains and pieces of the filter paper.

25-X. None pupated yet.
26-X, 4 P.M. Several pupated.
27-X, 2 P.M. 1 male and 1 female emerged.
29-X, noon. 2 females and 4 males emerged, all of good size and active. Probably a few emerged on 28-X, as judged by the number of dead mosquitos floating. No more larva or pupa left.

*Summary of observations on the life-history of* A. (S.) albopicta[1].

(*a*) The males were almost invariably the first to emerge.

(*b*) The minimum duration of the various stages was found to be approximately as follows :—

1. In the case of Pusa tree-hole materials :—

(i) At an average room temperature of about 21°C. :—
Incubation period, 18 hours (actually, probably less).
Larval priod, 8 days.
Pupal period, 3 days.

(ii) In incubator kept at 28°C. :—
Incubation period, 18 hours (actually, probably much less)
Larval period, 4 days.
Pupal period, 2 days.

2. In the case of mosquitos reared from eggs deposited in captivity :—

(i) At an average temperature of about 21°C. :—
Pre-oviposition period,[2] 7 days.
Incubation period, 8 days.
Larval period, 12 days.
Pupal period, 5 days.

(ii) In incubator kept at 28°C. :—
Pre-oviposition period, 3 days.
Incubation period, 3 days.
Larval period, 6 days.
Pupal period, 2 days.

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[1] A few attempts were made to induce *A.* (*S.*) *thomsoni* to oviposit in captivity, but they were unsuccessful. It is of interest that a few individuals of this species almost always emerged from the Pusa tree-hole materials after all the individuals of *A.* (*S.*) *albopicta* had been bred out therefrom.

[2] The expression " pre-oviposition period " is used here to mean the period between the first feed of blood and oviposition. As will be seen from the schedule of experiments, this period varied considerably, and this was what was to be expected, since such variation may have not only occurred as a result of intrinsic individual differences, but oviposition may have been delayed as a direct consequence of delayed pairing, although males were introduced into the cage at the very start of each experiment.

# APPENDIX II.

## *Table of Meteorological Observations.*

| Date | June | | July | | August | | September | | October | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum | Minimum |
| 1 | 79·0 | 65·9 | 74·2 | 58·9 | 69·4 | 60·1 | 67·6 | 58·1 | 54·4 | 50·7 |
| 2 | 80·6 | 64·1 | 62·2 | 59·1 | 67·2 | 59·9 | 64·6 | 58·9 | 57·6 | 52·3 |
| 3 | 79·4 | 55·5 | 66·6 | 59·3 | 65·7 | 58·0 | 67·2 | 59·1 | 61·0 | 55·1 |
| 4 | 79·2 | 63·3 | 71·6 | 59·6 | 65·4 | 59·0 | 65·2 | 58·5 | 64·2 | 52·1 |
| 5 | 78·8 | 66·0 | 73·7 | 60·9 | 60·2 | 59·0 | 68·2 | 57·0 | No record. | |
| 6 | 80·5 | 67·1 | 68·6 | 59·3 | 60·2 | 56·5 | No record. | | 63·4 | 51·5 |
| 7 | 80·2 | 65·5 | 69·2 | 59·1 | 62·0 | 58·1 | 60·2 | 56·9 | 63·4 | 49·9 |
| 8 | 80·6 | 67·1 | 63·2 | 57·5 | 69·0 | 60·0 | 71·2 | 57·3 | 62·0 | 51·1 |
| 9 | 80·7 | 67·0 | 67·0 | 58·1 | 69·4 | 59·1 | 68·6 | 56·5 | 66·2 | 53·3 |
| 10 | 82·6 | 68·5 | 72·2 | 60·1 | 70·6 | 59·1 | 69·2 | 57·7 | 67·2 | 52·9 |
| 11 | 81·4 | 66·3 | 72·0 | 60·5 | 64·1 | 59·0 | 68·2 | 58·1 | 66·8 | 53·9 |
| 12 | 78·2 | 62·5 | 69·2 | 58·3 | 63·4 | 59·1 | 65·4 | 56·6 | 66·2 | 52·1 |
| 13 | 78·1 | 56·1 | 70·4 | 58·1 | 69·6 | 58·5 | 69·1 | 55·7 | 65·2 | 50·7 |
| 14 | 76·7 | 62·5 | 66·2 | 57·1 | 71·8 | 60·1 | 66·6 | 55·7 | 64·1 | 50·1 |
| 15 | 81·2 | 63·9 | 67·2 | 57·9 | 75·0 | 60·9 | 66·7 | 54·5 | 64·0 | 48·9 |
| 16 | 81·0 | 66·0 | 70·6 | 59·5 | 74·6 | 57·1 | 66·1 | 55·1 | 64·8 | 48·0 |
| 17 | 80·6 | 64·5 | 68·0 | 58·7 | Not available. | Not available. | 67·2 | 53·6 | 63·2 | 49·1 |
| 18 | 77·8 | 59·6 | 68·1 | 57·0 | ,, | ,, | 68·5 | 55·9 | 61·4 | 49·9 |
| 19 | Not available | Not available | 67·6 | 56·3 | ,, | ,, | 69·7 | 54·7 | 61·6 | 49·6 |
| 20 | 68·2 | 58·9 | 63·2 | 57·1 | ,, | ,, | 72·0 | 56·7 | 61·2 | 47·5 |
| 21 | 70·4 | 60·7 | 71·8 | 59·1 | ,, | ,, | 66·8 | 55·3 | 59·6 | 46·1 |
| 22 | 73·1 | 61·1 | 65·2 | 58·9 | ,, | ,, | 55·6 | 50·6 | Not available. | Not available. |
| 23 | 75·2 | 62·0 | 64·0 | 58·1 | 66·2 | 57·3 | 56·0 | 50·1 | 58·0 | 46·3 |
| 24 | 76·2 | 63·5 | 66·7 | 57·1 | 65·3 | 58·3 | 53·7 | 49·9 | 60·2 | 47·3 |
| 25 | 76·2 | 62·1 | 65·2 | 59·1 | 72·2 | 59·1 | 62·0 | 52·1 | 61·2 | 47·6 |
| 26 | 74·2 | 60·9 | 68·2 | 58·1 | 66·2 | 58·1 | 66·6 | 56·5 | 61·7 | 49·9 |
| 27 | 67·2 | 61·3 | 67·7 | 58·9 | 68·0 | 58·3 | 67·2 | 57·3 | 62·6 | 48·2 |
| 28 | 72·0 | 59·6 | 67·0 | 59·3 | 65·1 | 57·6 | 66·6 | 56·3 | 1·8 | 49·5 |
| 29 | 73·4 | 61·5 | 71·2 | 62·0 | 69·0 | 59·8 | 65·6 | 52·1 | 62·4 | 45·5 |
| 30 | 76·8 | 60·6 | 69·4 | 59·9 | 62·7 | 57·7 | 59·2 | 50·5 | Experiments discontinued. | |
| 31 | Not available. | Not available. | 69·4 | 60·1 | 67·2 | 58·3 | Not available. | Not available. | .. | .. |

## PART II.

### Transmission of rinderpest by means of *Musca domestica*, Linn.

Few attempts appear to be on record to incriminate *Musca domestica* as an actual, as opposed to potential, carrier of disease. The charges against this fly would frequently appear to be based on evidence of a circumstantial character, there being almost complete lack of *data* on actual transmission experiments. *Primâ facie*, the indoor habits of the fly, and particularly the extent of access which it enjoys to almost every conceivable kind of food or filth, strongly suggest the possibility of these acting as intermediaries in the propagation of such common diseases as typhoid, cholera and dysentery. On the other hand, from a quantitative point of view, the numerical strength of the flies on the scene of the outbreak of a disease would frequently appear to be far in excess of the degree of virulence of the epidemic, and from this one would infer that the virulence of an epidemic does not, at any rate, proceed *pari passu* with the abundance of the flies. As a matter of fact, in the case of such diseases as are capable of being transmitted mechanically, there should be, obviously, at least more than one avenue of infection, and, as such, the *rôle* of flies in the mechanical transmission of such diseases should be, *a priori*, very limited.

Leaving aside the very numerous remarks of an *ex cathedrâ* character on the general complicity of *M. domestica* in the propagation of diseases, even the observations on the connexion of *M. domestica* with specific diseases (with the exception of habronemiasis) do not appear to be based on absolutely unequivocal grounds. In the case of such diseases as summer diarrhœa, dysentery, cholera and typhoid, no transmission experiments with *M. domestica* have been, for obvious reasons, ever attempted, and the possibility of the flies acting as intermediaries has been frequently solely deduced from the occurrence of the causative organisms in the invertebrate hosts, either on the surface of their body or within their intestinal tracts. Graham-Smith (1914) and Hewitt (1914) have adduced copious evidence of the presence of the causative organisims in the body of the invertebrate hosts, but very scanty reference occurs in their writings to actual transmission experiments having been carried out with *Musca*.

Coming to more recent works, reference may be made to the observations of Kling and Levaditi [1], who carried out a series of experiments on the fly transmission of poliomyelitis and obtained entirely negative results. Similar negative results were also obtained by Josefson, [2] although positive results ensued in a series of experiments carried out by Flexner and Clark, [3] but these were considered by Kling and Levaditi to be too artificial to be of value. The same remarks may

[1] *Ann. Inst. Pasteur*, XXVII, pp. 718-749 (1913). The article contains several important references.

[2] *L'Institut Med. de L'État*, Stockholm. III, p. 169 (1912) [Referred to by Kling and Levaditi. l.c., p. 729.]

[3] *Jl. Amer. Med. Assoc.*, LVI, pp. 1717-1718 (10 June 1911).

perhaps be applied to the results of inoculation experiments carried out by Howard and Clark, [1] although they demonstrated the presence of the poliomyelitis virus in an active state for several days on the surface of the body and for several hours in the intestinal tract of *M. domestica*.

The experiments of Lebœuf [2] on the fly transmission of leprosy enabled him merely to demonstrate the presence of Hansen's bacilli in the flies and he *inferred* that tranmission through the agency of flies could possibly occur in the immediate neighbourhood of leprous patients. Bayon [3] is also of the opinion that the disease is usually communicated by immediate contact and that it is unlikely that an insect carrier is generally involved. Marchoux [4] found that infection occurred when the proboscides of the flies had only been recently soiled and also when bacilli from the intestine of the fly were introduced beneath the skin of rodents.

Messerschmidt [5] carried out a series of experiments in which twenty rabbits infected with "typhus [typhoid] bacilli" were confined in a cow-shed of 400 cubic feet containing approximately 800 flies. The results were all negative.

In testing the possibility of the fly transmission of yaws, Castellani "allowed *M. domestica* to feed (1) upon yaws material (scraping from slightly ulcerated papules), and (2) upon semi-ulcerated papules on the skin of these yaws patients. In both cases he was able to discover the *Spirochæta pertenue* in microscopic preparations made from the flies' mouth parts and legs. Furthermore, he allowed *M. domestica* to feed on yaws material (1 and 2 as above) and afterwards transferred them to scarified areas upon the eye-brows of monkeys. Of 15 monkeys thus experimented upon three developed yaws papules at the places which had been contaminated by the flies". [6] Bahr [7], however, has contested Castellani's view and points to the absence of correlation of the disease with the abundance of flies in certain infected localities in Ceylon.

Very recently, Lebailly [8] has published the results of a series of experiments on the possibility of the foot-and-mouth disease being spread through the agency of *M. domestica*. Cattle infected with the disease were housed in a stable adjacent to one containing healthy cattle, a small opening covered with wire-gauze serving as a channel of communication between them. Numbers of *Muscina stabulans* and *M. domestica* were introduced in both stables, the small opening permitting of their freely passing from one stable to another. Five experiments, each lasting 15 days, were carried out. The results were entirely negative.

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[1] *Jl. Exper. Med.*, XVI, pp. 850-859 (1912).

[2] *Ann. Hyg. Méd. Colon.*, Paris, XVII, pp. 177-197 (1914). [Abstract in *Rev. App. Entom*, B, II, pp. 79-80 (1914).] Also, *Bull. Soc. Path. Exot.*, V, pp. 860-868 (1912).

[3] *Ann. Trop. Med. & Parasit.*, IX, pp. 60-61 (1915).

[4] *Ann. Inst. Pasteur*, XXX, pp. 61-68 (1916).

[5] *Centralbl. Bakt. Parasit. u. Infekt.* 1. Abt. Orig., LXXIV, pp. 1-5 (1914).

[6] Nuttall and Jepson. *Reports to the Local Govt. Board on Public Health and Medical Subject.* New series 16, No. 4, pp. 13-41 (1909). [Cited by Herms in *Medical and Veterinary Entomology*, p. 182 (1915).]

[7] *Ann. Trop. Med. & Parasit.*, VIII, p. 677 (1915).

[8] *C. R. Acad. Sci.*, CLXXIX, pp. 1225-1227 (1924).

As notable instances of diseases, besides habronemiasis, proved to be capable of being transmitted through the agency of *M. domestica*, mention may be made of anthrax, surra and a plague-like disease caused by *Bacterium tularense*.

As to the possibility of the insect transmission of anthrax, Morris [1] showed that *M. domestica* was capable of transmitting infection to wounds in healthy animals after feeding on flesh infected with anthrax bacilli or on discharges from open swellings.

In the case of surra, Mitzmain [2] found that the disease could be transmitted by *M. domestica* if applied on the abraded skin of the experimental animals. As Mitzmain's observations on the fly transmission of surra would appear to be the only extensive ones on the actual transmission of a disease through the agency of *Musca*, a few passages from his writings will bear quotation :—

" It is presupposed that the method is correlated with a previous skin abrasion affording the channel of entrance for the pathogenic organisms. Consideration of skin abrasions occurring through injury or disease must include the mechanical openings effected by various ectoparasites. The latter include such forms, as the ticks, gad flies and stable flies. It is obvious that in the presence of Muscids, with mouths constructed for lapping contaminated products, unlimited possibilities for conveyance of surra from host to host present themselves.

" It was aimed to prove, first, that this fly (*M. domestica*) could harbour infective organisms and was determined satisfactorily by numerous dissections and injections of saline suspensions of abdominal contents of flies fed on the abraded tail of the surra-infected monkey.....

" Attempts were made to simulate the normal relationship of parasitism in *M. domestica* and *S. calcitrans* by placing many flies of the two species in a common bottle and permitting them to attack the enclosed tail of a surra-infected monkey. Only laboratory-bred flies were employed.

" The five experiments were followed by negative results, demonstrating that under these conditions *M. domestica* does not transport infection through the channel afforded by the wound caused by the skin-piercing apparatus of *S. calcitrans*

" The possibility of surra infection being carried by the fly's feet was tested also. This was performed in the same manner as in the preceding, but with this difference : The infected flies were introduced from a separate bottle and by careful manipulation of the wire support attached to the monkey's tail the flies were permitted to alight on the appendage but were prevented from feeding.

" The five experiments failed to demonstrate that the wound made by the labium of the stable fly was a suitable channel for the introduction of trypanosomes carried on the pulvilli of *M. domestica*.

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[1] *Jl. Amer., Vet. Med. Assoc.* LVI, pp. 606-608 (1920).
[2] *New Orleans Medical and Surgical Journal*, LXIX, pp. 416-424 (1916-17).

"Finally, to serve as controls to these experiments, four tests were made to decide the question of the possibility of the punctures caused by the bite of the stable fly serving as sites for the introduction of trypanosomes in virulent blood. The four guinea-pigs used for the fly biting and the subsequent rubbing in of blood conveyed on a platinum loop escaped infection.

"The practical significance of the conveyance of trypanosomes obtained by *M. domestica* from the bite of the probing of the stable fly when wounds are present were finally investigated. Monkeys and horses were employed in this series. The house flies, after apparent engorgment of blood, derived from the probes of stable flies were transferred to clean bottles and the abraded surface of monkey's tail presented for the completion of the meal. The two horses exposed were scarified by scratching the haunch with a scalpel. Four of the five experiments attempted resulted in positive transmission."

With regard to *Bacterium tularense*, the causative organism of a plague-like disease in rodents, Wayson [1] found that washings of stable flies in normal salt-solution, when injected subcutaneously, produced positive results. "House flies used as mechanical porters in several trials proved capable of transferring infection from diseased viscera to healthy guinea-pigs. The bacteria were carried by the flies in their digestive tracts, mouthparts and dejecta, when immediately transferred to abrasion upon healthy rodents. Evidently the pulselli of contaminated house flies proved negligible as vehicles of infection. In four of the successful experiments flies were allowed to crawl on infected viscera, then induced to crawl on healthy conjuntiva prepared by cocanization and traumatized by rubbing sterile sand between the ocular and palpebral conjunctiva. This resulted, after 48 hours, in a purulent conjunctivitis, and after five to nine days, the death of the animals with cervical adenitis and typical lesions in the viscera."

From the foregoing references it would appear that although it is customary to incriminate the house-fly as an agent in the transmission of many diseases, particularly where bacterial as opposed to protozoal organisms are concerned, the actual *rôle* played by these insects in the propagation of diseases, under natural conditions, is very limited. Even in the case of such diseases as anthrax and surra, which have been definitely proved to be capable of being transmitted through the agency of *M. domestica*, such transmission has been shown to be only possible in the immediate neighbourhood of the infected animals. It would appear that the organisms, when ingested by the flies, remain viable usually only for a limited period within their alimentary tracts and rapidly attenuate whilst on the surface of the body of the flies. Bahr [2] found that the dysentery organism completely perished by the fifth day after its ingestion by the flies; nor was he able to obtain any indication of the multiplication of these organisms in the body of the fly. Experiments

[1] *Public Health Reports*, XXIX, pp. 3390-3393. [Abstract by M. B. Mitzmain in *Amer. Journ. Trop. Dis. & Prevent. Med.*, II, pp. 543-544 (1914-15).]
[2] *Brit. Med. Jl.*, pp. 294-296 (7 Feb. 1914).

conducted by Marchoux (*ante*) on the transmission of leprosy showed that the bacilli dried up whilst on the feet or proboscides of infected flies, and according to him the deposition of the bacilli along with excrement is of less common occurrence than is usually believed. In discussing the *rôle* of *M. domestica* in the propagation of amœbic dysentery and other intestinal protozoa, Roubaud [1] refers to the results of a series of experiments to prove that *M. domestica* can act as vector of certain intestinal protozoa under very limited conditions, particularly when their infected excrement is deposited on liquid or moist food. Root's [2] observations on the carriage of intestinal protozoa of man also point to the same conclusions, whilst Wollman [3] observed that flies infected with some of the common pathogenic organisms remained infective only for a few days. With regard to amœbic dysentery, Jausion and Dekester [4] remark that "the *rôle* of these flies in the transmission of *Entamœba dysenteriæ* is very limited," whilst in regard to *Giardia intestinalis*, the same authors observe that "the small and resistant cysts are undoubtedly well adapted to carriage by flies, but this does not conclusively prove that the infection is propagated by these insects."

It was considered that a series of experiments with *M. domestica* would definitely determine the possibility of the mechanical transmission of rinderpest and might also furnish indication with regard to the nature of the causative organism of the disease. The possibility of mechanical transmission seemed also to be indicated by the peculiar habit of the flies which—as one could not fail to notice in the cow-sheds at Muktesar—frequently sucked up the buccal and nasal discharges of infected animals and at the next moment crawled right up the nostrils of the animals and sometimes even regurgitated matter streaked with blood. The results of Shilston's experiments[5] on the vitality of the rinderpest virus would seem to indicate that the rinderpest organism is capable of causing the disease under conditions similar to those under which the virus may be transported through the agency of flies. A fair proportion of the present series of experiments was conducted with reference to the *data* recorded by Shilston, although the results were entirely negative.

## I. Methods and material.

The earlier experiments, which merely aimed at finding out whether *M. domestica* was capable of harbouring the causative organisms in a virulent form, were carried out by means of caught specimens, but in the subsequent series laboratory-bred flies only were employed. The flies were very numerous at Muktesar during the months of May and June, when they constituted almost a pest in the dwelling-

[1] *Bull. Soc. Path. Exot.*, XI, pp. 166-171 (1918).
[2] *Amer. Jl. Hyg.*, I, pp. 131-153 (1921). [Abstract in *Rev. App. Entom.*, B, IX, p. 126 (1921).]
[3] *C. R. Acad. Sci.*, CLXXII, pp. 298-301 (1921). Also, *Ann. Inst. Pasteur*, XXXV, pp. 431-449 (1921).
[4] *Arch. Insts. Pasteur Afr. Nord*, III, pp. 154-155 (1923). [Abstract in *Rev. App. Entom.*, B, XI, p. 173 (1923).]
[5] *Mem. Dept. Agri. India, Vet. Ser.*, III, No. 1 (October 1917).

houses and cow-sheds. The nuisance continued almost unabated until the onset of the Rains when the flies gradually became less numerous, but seemed to increase again towards the middle of September and steadily decreased with the advent of the cold weather.

The specific identity of the insect vector is obviously of much less importance in the case of diseases considered to be mechanically transmitted than in the case of diseases where a developmental cycle of the causative organism in a specialized environmental host is supposed to be involved. In experimenting with any species of *Musca*, however, it would seem to be of importance to determine whether it represents one of the true indoor species (*e.g.*, *M. domestica*, *M. vicina* and *M. nebulo*) or one of the many hæmatophagous Muscids. The intermittent habit of feeding of hæmatophagous Muscids has caused them to be suspected as carriers of various diseases (such as murrina), [1] but as this presupposes the existence of wounds to serve as portals of entry of the parasites, the *rôle* of such flies in the propagation of diseases can only be very limited and they can enter as a factor of only minor importance in any extensive epidemic. On account of their much less specialized feeding habits, the indoor species of *Musca*, such as *M. domestica*, would seem to be more likely to transmit diseases mechanically, such as by settling on the mucous membrane or on the conjunctiva or by depositing excrement on to fodder.

In the present series of experiments, the flies were carefully compared with the description of the Oriental species of *Musca* as published by Patton, and were identified as *M. domestica*.

For the purpose of rearing, caught flies were confined in cages made of mosquito-netting, and fresh horse manure, collected almost daily in the early morning, was offered to the enclosed flies for oviposition, the manure being placed on sheets of paper spread on the bottom of the cages. At first, both sugar solution, as recommended by Patton and Cragg (*l.c.*, p. 345), and milk, as recommended by Dunn[1] were offered as food to the flies, but later on, only milk was used as being more handy and much less sticky, and the flies fell to it with apparent avidity and quickly engorged themselves with the material.

The manure was daily removed from the cages and examined for eggs, and when found, these were removed to small glass jars and placed on fresh manure for the emerging larvæ to feed upon. The larvæ were daily supplied with fresh manure and the deposition of mould on the manure was carefully avoided either by sunning the jars or by speedily removing, by means of forceps, any trace of mould that had just begun to form.

In accordance with the method recommended by Patton and Cragg (*l. c.*, p. 344), the jars containing full-grown larvæ were placed in a mud enclosure containing a thick layer of earth on its bottom, in order that the larvæ that would crawl out of the jars might find, in the course of their migration, a ready place in the earth for

[1] Patton and Cragg. *A Text-book of Medical Entomology*, p. 733 (1913).
[2] *Bull. Ent. Res.*, XIII, pp. 301-305 (1923).

pupation. Experience showed, however, that, so far as this species of fly was concerned, the precaution was unnecessary, as the larvæ readily pupated within the jars, on the manure itself.

With the advent of the cold weather, metamorphosis was found to be much retarded under ordinary atmospheric conditions and recourse was therefore had to an incubator maintained at about 25°-30° C. for breeding the flies.

Three kinds of infective material were used, *viz.*, virulent blood, nasal discharge and fresh fæces, the materials being obtained from the animals at the height of the disease. A fresh supply of virulent blood was always in readiness at the Muktesar laboratory and the nasal discharge was obtained by swabbing the nostrils of infected animals by means of sterile swabs, whilst the small quantity of fæces that was needed could generally be obtained by gently scooping out matter from the anal meatus of affected animals.

For feeding the flies on blood, they were confined singly in tubes and the latter inverted over drops of blood placed on glass sheets, the tubes being gently tapped, if necessary, to bring the flies in contact with the blood. As this method frequently resulted in the flies, and sometimes the tubes themselves, being soiled with the infective material, a distinct improvement was effected when a piece of wire-gauze was made to intervene between the tube and the drop of blood. The gauze was kept gently pressed on the blood and a clean feed was usually secured by this method.

The necessity of the laborious process of feeding the flies singly arises, however, when in massive feeding it is dfficult to vouschafe whether any particular individual has fed or not. As the blood shows up well in the abdomen of an individual engorged with this material, the flies were usually fed on blood *en masse*, in bottles, except when individual feeding was specially called for for the purpose of any particular experiment.

For the reasons indicated above, the flies were fed singly when the infective material used was either nasal discharge or fæces ; and as it was exceedingly difficult from the mere appearance of the abdomen, to obtain any indication as to whether an individual had fed or not, a careful and sustained watch was kept to make sure that it actually fed[1].

In feeding the flies *en masse* on blood, they frequently very persistently confined themselves to the bottom of the inverted bottle which enclosed them, and would not migrate to have a feed of blood unless kept hungry almost to the point of death. Under such conditions, advantage was taken of their heliotropic tendency by covering the bottle with a roll of pasteboard and placing the glass sheet containing the infective material on the top of the cloth cage. This cut off a considerable portion of the light but left the glass sheet sufficiently illuminated to induce the flies to settle on the blood.

[1] Unless, however, it is intended to test the infectivity of the vomit or the excrement of the infected flies, it is obviously immaterial whether the flies have actually ingested the infected material or not, as the mere contact of the proboscis with the infective material should be sufficient for the purpose of investigating the possibility of a mechanical transmission.

The transmission experiments were carried out as follows [1] :—

(1) The flies were fed on virulent blood crushed on sterile mortar and inserted into pockets prepared beneath the skin of the experimental animals, in the region of the shoulders or the abdomen, the incision being subsequently stitched up.

(2) The flies were crushed in sterile normal saline solution, the emulsion filtered through a piece of wiregauze and the filtrate injected intravenously.

(3) Living infected flies were brought in contact with wounds, nasal mucous membrane and conjunctiva.

(4) Washings of infected flies were smeared on wounds, nasal mucous membrane and conjunctiva.

(5) Crushed feet and proboscides of infected flies were smeared on nasal mucous membrane and conjunctiva.

(6) Saline suspensions of infected materials were administered by drenching, or entire flies were given through the medium of fodder, the object being to test the receptivity of the buccal or the gastric mucous membrane for the causative organism. In this connection reference may be made to the fact that, according to Strickland,[2] rats may become infected with *Trypanosoma lewisi* by swallowing the invertebrate host (the rat flea) of this protozoon.

For collecting excrement, the infected flies were confined in sterile glass tubes and the fly " specks " deposited on the walls of the tubes were emulsified in normal saline solution with the aid of a sterile swab.

The incisions in the experimental animals were made by means of a sterile scalpel. At first, a dozen superficial incisions were made, but this resulted in a somewhat copious flow of blood which, on coagulation, seemed to block up the incisions. Later on, therefore, one or two superficial incisions only were made, these being gently opened afresh in the course of the experiments when the blood showed tendency to coagulate.

In bringing the flies in contact with the nasal mucous membrane and conjunctiva, pieces of mosquito-netting were found more convenient than glass tubes for enclosing the flies.

The interval between the infective feed and the contact of the infective flies with the experimental animals varied from one minute to about four hours. In the case of very short intervals, the flies were always fed singly and not allowed to have a full feed of the virulent blood (which was the only material used in such experiments) in order that they might settle readily on the abraded skin.

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[1] To kill the flies, chloroform was employed.
[2] Cited by Patton and Cragg. *L.c.*, p 736.

In the case of long intervals, the flies were kept enclosed in bottles, and to ensure a good supply of moisture moistened rags were kept hung up from the cotton wool that plugged the mouth of the bottle. In the later experiments, however, bottles were discarded in favour of cloth cages for confining the infected flies, as the inside walls of the bottles were sometimes found much bedewed with moisture which caused the flies to get stuck up or otherwise damaged. The flies were allowed feeds of milk but as a rule they were kept fasting as long as possible.

## II. Schedule of experiments.

*Series I. Subcutaneous insertion of crushed flies. Virulent blood used as infective material. Wild flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 177 (Chupper 21) | 161 | 11 | 14-V-24 | 26 hours | Positive | The flies included 2 dead ones. |
| 2 | 177 (Chupper 21) | 174 | 22 | 17-V-24 | 24 hours | | |
| 3 | 177 (Chupper 21) | 170 | 23 | 21-V-24 | 31 hours | | The flies included 5 dead ones. |
| 4 | 178 (Chupper 22) | 174 | 22 | 15-V-24 | 24 hours | Negative | |
| 5 | 178 (Chupper 22) | 176 | 10 | 18-V-24 | 24 hours | | |
| 6 | 239 (Chupper 61) | 176 | 23 | 20-V-24 | 52 hours | Negative | All the flies were dead ones |

*Series II. Intravenous inoculation of saline suspensions (in normal saline solution) of crushed flies.*

*Virulent blood used as infective material. Wild flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 246 (Chupper 24) | 223 | 16 | 22-V-24 | 3 hours | Positive | Bull recovered |
| 2 | 246 (Chupper 24) | 86 | 7 | 24-V-24 | 3 hours | | |
| 3 | 248 (Chupper 27) | 223 | 13 | 23-V-24 | 3 hours | Positive | |
| 4 | 268 (Chupper 39) | 16 | 14 | 31-V-24 | 3 hours | Positive | |
| 5 | 267 (Chupper 38) | 16 | 15 | 31-v-24 | 6 hours | Positive | |
| 6 | 267 (Chupper 38) | 19 | 18 | 2-vi-24 | 6 hours | | |
| 7 | 331 (Chupper 43) | 2 | 15 | 1-vi-24 | 7 hours | | The animal died of hæmorrhagic septicæmia. |
| 8 | 331 (Chupper 43) | 17 | 20 | 3-vi-24 | 6½ hours | | |

*Series II. Intravenous inoculation of saline suspensions (in normal saline solution) of crushed flies*—contd.

*Virulent blood used as infective material. Wild flies employed*—contd.

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 9 | 265 (Chupper 34) | 93 | 20 | 28-v-24 | 12 hours | Positive | |
| 10 | 265 (Chupper 34) | 16 | 12 | 31-v-24 | 12 hours | | |
| 11 | 266 (Chupper 35) | 89 | 20 | 29-v-24 | 12 hours | Positive | |
| 12 | 264 (Chupper 36) | 20 | 16 | 30-v-24 | 12 hours | Negative | |
| 13 | 255 (Chupper 31) | 223 | 14 | 24-v-24 | 27 hours | ? Positive | |
| 14 | 255 (Chupper 31) | 92 | 14 | 27-v-24 | 24 hours | | |
| 15 | 254 (Chupper 29) | 86 | 15 | 25-v-24 | 24 hours | Negative | The flies included 6 dead ones. |
| 16 | 254 (Chupper 29) | 74 | 17 | 26-v-24 | 24 hours | | |
| 17 | 247 (Chupper 28) | 75 | 12 | 23-v-24 | 50 hours | Negative | The flies included only 2 live ones. |
| 18 | 247 (Chupper 28) | 92 | 22 | 29-v-24 | 48 hours | | |
| 19 | 247 (Chupper 28) | 16 | 15 | 2-vi-24 | 50 hours | | |
| 20 | 269 (Chupper 37) | 89 | 12 | 31-v-24 | 51 hours | Negative | The flies included many dead ones. |
| 21 | 269 (Chupper 37) | 19 | 12 | 4-vi-24 | 48 hours | | The flies included 7 or 8 either dead or moribund. |

*Series III. Intravenous inoculation of saline suspensions (in n. s.s.) of crushed flies.*

*Nasal discharge used as infective material. Wild flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 347 (Chupper 20) | 328 | 22 | 7-vi-24 | 3 hours | Positive | |
| 2 | 347 (Chupper 20) | 110 | 18 | 10-vi-24 | 3 hours | | |
| 3 | 349 (Chupper 18) | 169 | 11 | 9-vi-24 | 3 hours | Positive | |
| 4 | 349 (Chupper 18) | 338 | 21 | 13-vi-24 | 3 hours | | |
| 5 | 350 (Chupper 19) | 328 | 23 | 8-vi-24 | 24 hours | Negative | Only 7 of these flies were alive. |
| 6 | 350 (Chupper 19) | 110 | 12 | 12-vi-24 | 24 hours | | The flies included 2 dead ones. |
| 7 | 352 (Chupper 17) | 110 | 14 | 11-vi-24 | 24 hours | Negative | Only 2 of these flies were alive. |
| 8 | 353 (Chupper 17) | 338 | 22 | 15-vi-24 | 24 hours | | The flies included 10 dead ones |

*Series IV. Intravenous inoculation of saline suspensions (in n. s.s.) of crushed flies.*

*Fæces used as infective material. Wild flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 409 (Chupper 26) | 238 | 12 | 22-vi-24 | 3½ hours | Positive | |
| 2 | 409 (Chupper 26) | 178 | 23 | 25-vi-24 | 3 hours | | |
| 3 | 488 (Chupper 31) | 178 | 20 | 24-vi-24 | 3 hours | Positive | |
| 4 | 488 (Chupper 31) | 407 | 14 | 26-vi-24 | 3 hours | | |
| 5 | 400 (Chupper 15) | 120 | 9 | 18-vi-24 | 24 hours | Negative | All the flies were dead. |
| 6 | 400 (Chupper 15) | 226 | 12 | 20-vi-24 | 24 hours | | The flies included a dead one. |
| 7 | 400 (Chupper 15) | 163 | 14 | 24-vi-24 | 27 hours | | |
| 8 | 405 (Chupper 9) | 120 | 16 | 19-vi-24 | 24 hours | Negative | The flies included one dead and one moribund. |
| 9 | 405 (Chupper 9) | 238 | 12 | 21-vi-24 | 24 hours | | The flies included 2 dead ones. |

*Series V. Smearing of crushed legs and proboscides (in n. s.s.) of infected flies on scarified skin.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 340 (Chupper 41) | 348 | 27 | 30-vi-24 | 4 hours | Negative | |
| 2 | 340 (Chupper 41) | 269 | 27 | 1-vii-24 | 3 hours | | |

*Series VI. Smearing of crushed legs and proboscides (in n. s.s.) on nasal mucous membrane and conjunctiva.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 334 (Chupper 46) | 348 | 29 | 30-vi-24 | 4 hours | Negative | |
| 2 | 334 (Chupper 46) | 269 | 27 | 1-vii-24 | 3 hours | | |

*Series VII. Intravenous inoculation of saline suspensions (in n.s.s.) of crushed legs and proboscides.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 335 (Chupper 47) | 348 | 29 | 30-vi-24 | 4 hours | Negative | |
| 2 | 335 (Chupper 47) | 269 | 27 | 1-vii-24 | 3 hours | | |

*Series VIII. Smearing of washings (in n. s.s.) of infected flies on scarified skin.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 341 (Chupper 48) | 348 | 36 | 30-vi-24 | 4½ hours | Negative | |
| 2 | 341 (Chupper 48) | 269 | 22 | 1-vii-24 | 3 hours | | |

*Series IX. Smearing of washings (in n. s.s.) of infected flies on nasal mucous membrane and conjanctiva.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 342 (Chupper 49) | 348 | 36 | 30-vi-24 | 4½ hours | Negative | |
| 2 | 342 (Chupper 49) | 269 | 22 | 1-vii-24 | 3 hours | | |

*Series X. Intravenous inoculation of washings (in n. s.s.) of infected flies.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Exprimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 343 (Chupper 50) | 348 | 36 | 30-vi-24 | 4½ hours | Negative | |
| 2 | 343 (Chupper 50) | 269 | 22 | 1-vii-24 | 3 hours | | |

*Series XI. Smearing of excrement (in n. s.s.) of infected flies on scarified skin.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 344 (Chupper 52) | 490 | 39 | 2-vii-24 | 3 hours | Negative | About 13 fly "specks" which included several blood spots. |
| 2 | 344 (Chupper 52) | 269 | 61 | 3-vii-24 | 3 hours | | |

*Series XII. Smearing of excrement (in n. s.s.) of infected flies on nasal mucous membrane and conjunctiva.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of smearing | Interval between infective feed and smearing | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 345 (Chupper 53) | 490 | 39 | 2-vii-24 | 3 hours | Negative | About 13 fly "specks" which included several blood spots. |
| 2 | 345 (Chupper 53) | 269 | 61 | 3-vii-24 | 3 hours | | |

*Series XIII. Intravenous inoculation of saline emulsion of excrement of infected flies.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of inoculation | Interval between infective feed and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 346 (Chupper 5 ) | 490 | 39 | 2-vii-24 | 3 hours | Negative | About 13 fly "specks" which included several blood spots. |
| 2 | 346 (Chupper 5 ) | 269 | 61 | 3-vii-24 | 3 hours | | |

*N. B.* About 40 fly "specks" including several blood spots were mixed with n. s. s. and one-third of this emulsion was used in each of the above three experiments.

*Series XIV. Oral administration (by drenching) of saline emulsion of excrement of infected flies.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of oral administration | Interval between infective feed and oral administration | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 415 (Chupper 29) | 522 | 12 | 7-vii-24 | 4½ hours | Negative | |
| 2 | 415 (Chupper 29) | 333 | 30 | 8-vii-24 | 4 hours | | |

*Series XV. Oral administration (by drenching) of washings (in n. s.s.) of infected flies.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of oral administration | Interval between infective feed and oral administration | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 249 (Chupper 27) | 522 | 18 | 7-vii-24 | 4½ hours | Negative | |
| 2 | 249 (Chupper 27) | 333 | 20 | 8-vii-24 | 4 hours | | |

*Series XVI. Contact of living infected flies with nasal mucous membrane or conjunctiva or both.*

*Virulent blood used as infective material. Laboratory-bred flies employed.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of contact | Interval between infective feed and contact | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 339 (Chupper 24) | 490 | About 25 | 4-vii-24 | 3 hours | Negative | Contact could not be prolonged as the flies escaped owing to bull being turbulent. |
| 2 | 339 (Chupper 24) | 336 | Not noted | 5-vii-24 | 3 hours | | |
| 3 | 339 (Chupper 24) | 336 | 12 | 6-vii-24 | 3 hours | | |
| 4 | 429 (Chupper 9) | 530 | 30 | 11-vii-24 | 3½ hours | Negative | |
| 5 | 429 (Chupper 9) | 400 | 12 | 12-vii-24 | 2½ hours | | |

*Series XVII. Contact of living infected flies with scarified skin.*

*Virulent blood used as injective material. Laboratory-bred flies employed.*
*Result : Negative.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of contact | Interval between infective feed and contact | Remarks |
|---|---|---|---|---|---|---|
| 1 | 1036 (Chup. 30) | 490 | About 35 | 4-vii-24 | 3 hours | The number of Experimental Bull represents number on horn. |
| 2 | 1036 (Chup. 39) | 336 | Not noted | 5-vii-24 | 3 hours | |
| 3 | 1036 (Chup. 39) | 336 | 18 | 6-vii-24 | 3 hours | |
| 4 | 488 (Chup. 33) | 405 | 10 | 17-vii-24 | 3½ hours | |
| 5 | 488 (Chup. 33) | 403 | About 20 | 18-vii-24 | 3 hours | |
| 6 | 563 (Chup. 32) | 556 | 4 | 24-vii-24 | 1 hour | |
| 7 | 563 (Chup. 32) | 559 | 8 | 25-vii-24 | 1 hour | |
| 8 | 563 (Chup. 32) | 559 | 12 | 26-vii-24 | 1 hour | |
| 9 | 563 (Chup. 32) | 558 | 13 | 27-vii-24 | 1½ hours | |
| 10 | 563 (Chup. 32) | 558 | 15 | 28-vii-24 | 1 hour | |
| 11 | 563 (Chup. 32) | 556 | 7 | 29-vii-24 | 1½ hours | |
| 12 | 563 (Chup. 32) | 334 | 22 | 30-vii-24 | 1½ hours | |
| 13 | 330 (Chup. 52) | 340 | 19 | 31-vii-24 | 15 minutes | |
| 14 | 330 (Chup. 52) | 341 | About 17 | 1-viii-24 | 15 minutes | |
| 15 | 330 (Chup. 52) | 342 | About 17 | 2-viii-24 | 15 minutes | |
| 16 | 330 (Chup. 52) | 343 | About 8 | 3-viii-24 | 15 minutes | |
| 17 | 330 (Chup. 52) | 343 | About 9 | 4-viii-24 | 15 minutes | |
| 18 | 1184 (Stable 1) | 562 | 3 | 17-viii-24 | 1 to 2 minutes | The number of Experimental Bull represents number on horn. |
| 19 | 1184 (Stable 1) | 562 | 9 | 18-viii-24 | 1 to 2 minutes | |
| 20 | 1184 (Stable 1) | 560 | 12 | 19-viii-24 | 1 to 2 minutes | |
| 21 | 1184 (Stable 1) | 560 | 9 | 20-viii-24 | 1 to 2 minutes | |
| 22 | 855 (Chup. 2) | 590 | 9 | 29-viii-24 | 1 to 2 minutes | |
| 23 | 855 (Chup. 2) | 657 | 9 | 3-ix-24 | 1 to 2 minutes | |
| 24 | 855 (Chup. 2) | 688 | 5 | 7-ix-24 | 1 to 2 minutes | |
| 25 | 855 (Chup. 2) | 674 | 5 | 8-ix-24 | 1 to 2 minutes | |
| 26 | 855 (Chup. 2) | 688 | 10 | 9-ix-24 | 1 to 2 minutes | |
| 27 | 855 (Chup. 2) | 832 | 2 | 10-ix-24 | 1 to 2 minutes | |
| 28 | 855 (Chup. 2) | 697 | 8 | 13-ix-24 | 1 to 2 minutes | |
| 29 | 855 (Chup. 2) | 870 | 16 | 14-ix-24 | 1 to 2 minutes | |
| 30 | 855 (Chup. 2) | 912 | 15 | 15-ix-24 | 1 to 2 minutes | |
| 31 | 855 (Chup. 2) | 693 | 17 | 19-ix-24 | 1 to 2 minutes | |

*Series XVIII. Contact of living infected flies with nasal mucous membrane or conjunctiva or both.*

*Fæces used as infective material. Laboratory-bred flies employed. Result : Negative.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of contact | Interval between infective feed and contact | Remarks |
|---|---|---|---|---|---|---|
| 1 | 253 (Chup. 44) | 558 | 24 | 28-vii-24 | 1 hour | |
| 2 | 253 (Chup. 44) | 564 | 26 | 30-vii-24 | 1 hour | |
| 3 | 253 (Chup. 44) | 564 | 30 | 31-vii-24 | 1 hour | |
| 4 | 253 (Chup. 44) | 558 | 31 | 1-viii-24 | 1 hour | |
| 5 | 253 (Chup. 44) | 334 | 15 | 2-viii-24 | 1 hour | |
| 6 | 253 (Chup. 44) | 342 | 18 | 3-viii-24 | 1 hour | |
| 7 | 253 (Chup. 44) | 342 | 3 | 4-viii-24 | 1 hour | There were two more flies used in this expt. These had soiled themselves with infective material but were unfed. |

*Series XIX. Oral administration of entire infected flies (alive or killed immediately before administration) through the medium of fodder.*

*Virulent blood used as infective material. Laboratory-bred flies employed. Result : Negative.*

| Serial No. | Experimental bull No. | Control No. | Number of flies | Date of oral administration | Interval between infective feed and oral administration | Remarks |
|---|---|---|---|---|---|---|
| 1 | 405 (Chup. 30) | 333 | 11 | 9-vii-24 | 3 hours | |
| 2 | 405 (Chup. 30) | 530 | 12 | 10-vii-24 | 3 hours | |
| 3 | 321 (Chup. 41) | 559 | 12 | 26-vii-24 | 1 hour | |
| 4 | 321 (Chup. 41) | 558 | 13 | 27-vii-24 | 2 hours | |
| 5 | 321 (Chup. 41) | 558 | 15 | 28-vii-24 | 1½ hours | |
| 6 | 321 (Chup. 41) | 556 | 7 | 29-vii-24 | 1½ hours | |
| 7 | 321 (Chup. 41) | 334 | 20 | 30-vii-24 | 1½ hours | |
| 8 | 321 (Chup. 41) | 340 | 14 | 31-vii-24 | 45 minutes | |
| 9 | 321 (Chup. 41) | 341 | 15 | 1-viii-24 | 1 hour | |
| 10 | 321 (Chup. 41) | 342 | 17 | 2-viii-24 | 30 minutes | |
| 11 | 321 (Chup. 41) | 343 | 8 | 3-viii-24 | 30 minutes | |
| 12 | 321 (Chup. 41) | 343 | 10 | 4-viii-24 | 30 minutes | |

## PART III.

### Transmission of rinderpest by means of the Cattle Louse, *Linognathus vituli*, Linn.

Although it was recognized that, from an epidemiological point of view, there were only very remote possibilities of rinderpest proving to be a louse-borne disease under natural conditions, it was considered that, in view of the existence of an immunological similarity between this disease and typhus,[1] the results of a series of transmission experiments by means of the common cattle louse might furnish indication as to the probable character of the causative organism of the disease.

#### I. Methods and material.

The experiments, which were of a very preliminary character, were designed with reference to the recent findings of Bacot and Ségal [2], and Atkin and Bacot, [3] regarding the conveyance of *Rickettsia prowazeki* by the body-louse, *Pediculus humanus*. Only seven experiments, divided into two series, were carried out :—

*Series I.* The lice were collected from infected animals, crushed on sterile mortar in sterile normal saline solution and the emulsion was injected intravenously into healthy animals.

*Series II.* The lice were collected from infected animals and transplanted alive on healthy animals, the object being to ascertain whether the disease could be caused through the bites of infected lice. So far as typhus is concerned, Bacot and his collaborators would seem to discredit the possibility of the disease being caused through the bites of infected lice and the results of their investigations would appear to indicate that it is in reality the excrement of infected lice that is infective, although they have hitherto been unable to advance a hypothesis as to the channel by which the parasites thus voided find their entry into the tissues of the vertebrate host. The existence of a previous abrasion to serve as a site for the introduction of the parasites can only be exceptional and can hardly be regarded as a factor of any importance in the ætiology of the disease. In the case of the louse-borne relapsing fever, it is generally agreed – and the hypothesis is supported by experimental evidence —that infection results from the crushing of the infected lice, whereby the liberated parasites usually find their entry through the epidermis beneath the nails. *Primâ facie*, this explanation would appear to be the only possible one, inasmuch as the spirochætes, once they are locked up in the haematocoele, can only be released as a result of the rupture of the invertebrate host. In the case of typhus, however, such a hypothesis would seem to be hardly indicated, since the punctures inflicted by the lice themselves may serve as portals of entry of the causative organisms voided with

[1] *Report of the Imperial Bacteriological Laboratory, Muktesar, for the two years ending the 31st March, 1924*, pp. 35-36 (1925).

[2] *Brit. Jl. Exptl. Pathology*, III, pp. 125-132 (1922).

[3] *Brit. Jl. Exptl. Pathology*, III, pp. 196-203 (1922). Also, other papers by Bacot and his collaborators.

the excrement. This possibility has been emphasized by Patton and Cragg [1] who observe : " Exit of the parasite per annum is probably a commoner and is certainly a more easily understood event. Infection in this case is dependent on the well-known habit of defaecation at the time of feeding, or immediately after it, which is to be observed in the great majority of blood-sucking insects. The excrement thus voided is of course in close approximation to the wound made by the mouthparts, and may mingle with the drop of blood which oozes out when the proboscis is withdrawn, so that there is ample opportunity for the parasite, especially if it is motile, to pass into the tissues of the invertebrate [*sic*,? vertebrate] through the wound." As, however, such contact of the infective excrement with the wound is obviously very largely a matter of chance, the *data* advanced by Bacot and his collaborators do not appear to be sufficiently copious to preclude the possibility of the disease being caused through the bites of infected lice.

## II. Schedule of Experiments.

*Series I. Intravenous inoculation of saline suspensions (in n. s.s.) of crushed infected lice.*

| Serial No. | Experimental bull No. | Control No. | Number of lice | Date of inoculation | Interval between removal from Control and inoculation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 1183 (Stable 1) | 563 | 25 | 17-viii-24 | 30 minutes | Positive | The number of Experimental bull represents number on horn. 10 lice from Control 560; 25 lice from Control 671. |
| 2 | 1183 (Stable 1) | 570 | 25 | 18-viii-24 | 15 minutes | | |
| 3 | 1183 (Stable 1) | 560 and 671 | 125 | 19-viii-24 | 15 minutes | | |
| 4 | 10 (Stable 1) | 634 | 21 | 27-viii-24 | 3 hours | Negative | The number of Experimental bull represents number on horn. |
| 5 | 1072 | 915 | 25 | 17-ix-24 | 30 minutes | | |

*Series II. Transplantation of living lice.*

| Serial No. | Experimental bull No. | Control No. | Number of lice | Date of transplantation | Interval between removal from Control and transplantation | Result | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 868 (Chupper 3) | 682, 596 and 590 | 2+15+112 =129 | 1-ix-25 | 1½ hours | Negative | |
| 2 | 868 (Chupper 4) | 693 | 25 | 19-ix-25 | 30 minutes | | |

[1] *A Text-book of Medical Entomology*, p. 735 (1913).

**Summary.**

The transmission experiments recorded in this report resolve themselves into three categories :—

I. Transmission experiments by means of *Aëdes* (*Stegomyia*) *albopicta*.

These experiments were suggested by certain bacteriological findings obtained by the Director of the Imperial Institute of Veterinary Research, Muktesar, in the course of his investigations upon the ætiology of rinderpest, which seemed to indicate that the causative organism of the disease was, in many of its characteristics, probably not unlike *Leptospira icteroides*, the spiochæte incriminated with the causation of yellow fever and known to be transmitted by *A.* (*S.*) *argenteus*.

The results of the experiments on hill bulls were negative, but some of the rabbits experimented upon showed thermal reactions. Attempts were made to intensify these reactions by "passaging" through a series of rabbits the blood obtained from the originally infected (bitten) rabbits, but although pronounced reaction frequently resulted, the results were vitiated by an outbreak of pasteurellosis amongst the experimental animals. In the schedule of experiments, therefore, only such clinical charts are exhibited as represent results obtained by feeding infected mosquitos on rabbits. The results of "passaging" are withheld until such time as the experiments may be repeated under conditions precluding all possibility of supervention of pasteurellosis.[1]

II. Transmission experiments by means of *Musca domestica*.

The experiments of this series were carried out with the object of testing the possibility of the mechanical transmission of rinderpest through the agency of the house-fly. In a fair proportion of the experiments, positive results were obtained when bodies of flies fed on infective materials (such as virulent blood, nasal discharge and fæces of infected animals) were inoculated into susceptible bulls; but the results were negative when the experiments were conducted with reference to the natural conditions under which the disease was likely to be transmitted through the agency of *M. domestica*.

III. Transmission experiments by means of *Linognathus vituli*.

A series of experiments with lice was suggested by the existence of an immunological similarity of rinderpest to human typhus, the exciting agent of which is known to be transmitted by *Pediculus humanus*. The infectivity of saline suspen-

[1] Recent researches at Muktesar Laboratory upon hæmorrhagic septicæmia have been directed with the object of evolving methods of securing products for combating the disease, and the anti-serum now obtainable, when administered in the dosage recommended, is capable of warding off a virulent fatal infection.

sions of crushed infected lice was tested upon three bulls and of these one developed rinderpest. The results of transplantation of infected lice on a healthy bull were negative.

References are made in this report to certain aspects of the inquiry which, although they have no direct bearing on entomology, are nevertheless included here in order that the whole question may be presented in its true perspective. These aspects relate particularly to the conceptions involved in the expressions "developmental cycle" and "intermediary host" and to certain important features of the work done on arthropods as vectors of spirochætes. A brief review of the work done on yellow fever is also given inasmuch as the first part of the inquiry was conducted chiefly on the analogy of this disease.

ACKNOWLEDGMENTS.

The writer desires to express his grateful thanks to Mr. J. T. Edwards, Director, Imperial Institute of Veterinary Research, Muktesar, for the many courtesies extended and for kindly criticism and much helpful guidance in the prosecution of the investigation.

The work of inoculation and "passaging" through rabbits was conducted by Mr. M. B. Menon, and the clinical observations on the experimental bulls were carried out under the supervision of Mr. J. D'Costa. To both these officers the writer's sincere thanks are due.

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